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Isozymes and forest trees: An annotated bibliography

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Isozymes and forest trees: an annotated bibliography

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Abstract

The bibliography contains references on forest isozyme research, with author, species and subject indices. It covers the period 1970-1985.

Résumé

La bibliographie renferme des références sur la recherche sur les isoenzymes des essences forestières, pour la période de 1970 à 1985. Elle comprend des index des auteurs, des essences et des matières.

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Foreword

In the past decade, extensive studies have been carried out using isozyme techniques on the genetic variation of forest trees. These studies dealt with genetic heterozygosity, population genetic structure, outcrossing rates, population dynamics, sampling strategies, correlations between allozyme genotypes and quantitative traits and the mode of inheritance and linkage relationships of allozymes. Isozyme research has been conducted on a world-wide basis, and the results often remain scattered and unavailable to tree geneticists.

This bibliography contains references on the current state of knowledge in forest isozyme research and highlights areas where more research is needed. Hopefully it will be of assistance in establishing research priorities for universities, research organizations and industry.

This bibliography contains 249 references to world literature on isozyme research in forest trees. In compiling it, Forestry Abstracts, Genetics Abstracts and Plant Breeding Abstracts were searched. We are grateful to these journals for permission to reproduce abstracts. References were also obtained from publication citations and from numerous authors through personal contacts.

The general format of the bibliography includes a listing of citations and of abstracts followed by author, species and subject indices. Citations are numbered consecutively; authors are listed both alphabetically and chronologically by senior author. Citations include author(s), publication date, title, journal or publication series, volume and page(s). The indices are included for ease in locating references by citation number.

We apologize to the authors of pertinent articles which have escaped our attention. Compilation of this bibliography ended in August, 1984.

Yousry A. El-Kassaby
Eleanor E. White
August, 1984.

Acknowledgements

We would like to thank Leila El-Kassaby for preparing the indices, J.B. Mitton and O. Sziklai for their encouragement, and colleagues who allowed access to their manuscripts before publication.

1. Adams, W.T. 1979.

Applying isozyme analyses in tree-breeding programs. *In: Proceedings of Symposium on Isozymes of North American Forest Trees and Insects*, July 27, 1979, Berkeley, Calif. Gen. Tech. Rep. PSW-48. Pacific Southwest Forest and Range Exp. Sta., Forest Serv., U.S. Dep. Agric., Berkeley, Calif., pp. 60-64.

Four examples illustrate the potential for practical use of isozyme analyses in applied breeding programs. These include identifying parent trees and clones, seed sources and parentage of controlled crosses, and evaluating the effectiveness of different procedures involving open-pollination to produce seed of specific crosses. The improved ability to assess the true identity of parents, seed stocks, and controlled crosses justifies the added expense resulting from the use of isozyme analyses in applied tree-breeding programs.

2. Adams, W.T. 1981.

Population genetics and gene conservation in Pacific Northwest conifers. *In: G.C.E. Scudder and J.L. Reveal (eds.), Evolution Today, Proceedings of the Second International Congress of Systematic and Evolutionary Biology*, pp. 401-415.

Development of efficient gene conservation strategies require information on population genetic structure. In this paper, current knowledge of population genetic structure in Pacific Northwestern conifers is reviewed briefly and the need for further study is emphasized. In general, these species possess a great deal of genetic diversity, both within and among populations. Furthermore, genetic variation among populations is often strongly correlated with changes in the physical environment. Such adaptational patterning of genetic variation poses great difficulties for gene conservation programs, since the number of adaptively different populations could be very large. The consequences of planting non-local seed sources, in terms of growth and survival under plantation management, await adequate appraisal. Until this appraisal is made, an effort must be made to maintain the genetic integrity of adaptively different

populations, so that gene pools possessing natural adaptations to different environments are not lost. Based on estimates of genetic diversity calculated from allozyme data, recently domesticated Douglas-fir populations do not appear to be appreciably less variable than natural populations in the same regions. This is attributable to procedures which emphasize a broad genetic base in breeding materials.

3. Adams, W.T. 1983.

Application of isozymes in tree breeding. *In: S.D. Tanksley and T.J. Orton (eds.), Isozymes in plant genetics and breeding, Part A*, pp. 381-400. Elsevier Science Publishers B.V. Amsterdam. (Review).

4. Adams, W.T. and S. Coutinho. 1977.

Isoenzyme genetic markers useful for studies of the *Pinus rigida* × *P. taeda* hybrid. *In: Proceedings, 24th Northeastern Forest Tree Improvement Conference*. Broomall, Pennsylvania, U.S.A.: Northeastern Forest Experiment Station, U.S.D.A. Forest Service, pp. 1-13.

Genetically determined isozyme markers useful for studies of the *Pinus rigida* × *P. taeda* (pitch × loblolly pine) hybrid are described. Seedling progeny from seven two-parent pitch × loblolly pine hybrid crosses, as well as seedling progeny from single species crosses involving each of the seven parents of these hybrids (parental families), were subjected to starch gel electrophoresis and stained for six different enzymes. Female gametophyte (1N) segregation patterns of isozymes in heterozygous parental families were used to determine the Medelian genetics of the band patterns. Band patterns of embryos in the parental families were used to determine the expression of isozymes in diploid tissue of each species. Band patterns were strikingly similar in the two species and in hybrids were exactly those predicted from the genotypes and phenotypes expressed in the parental families. Thus, for the six enzymes studied, hybrids cannot be distinguished from individuals of non-hybrid origin on the basis of uniquely "hybrid" band types.

However, individuals of hybrid origin are distinguishable when the parents of one species carry alleles leading to bands of different migration rate from those carried in the parents of the other species. Under these conditions, hybrid individuals express heterozygous phenotypes which are not possible among crosses within the species. Examples of how these markers may be used in genetic studies of this hybrid are given.

5. Adams, W.T. and R.J. Joly. 1980.

Allozyme studies in loblolly pine seed orchards: clonal variation and frequency of progeny due to self-fertilization. *Silvae Genet.* 29: 1-4.

As part of a study using allozymes to investigate factors associated with genetic efficiency in two adjacent loblolly pine (*Pinus taeda* L.) seed orchards, we wished to determine 1) the degree of allozyme variability among clones and 2) the proportion of orchard progeny resulting from self-fertilization. Considerable genetic variation was found among the 50 clones in the two orchards. Twelve of the 15 loci studied were polymorphic, averaging 3.4 alleles per locus; nearly 25% of the polymorphic loci were heterozygous in any one individual. The potential for using allozymes as clone marker in breeding programs is demonstrated by the fact that 47 of the 50 clones could be uniquely identified based on their allozyme genotypes.

Allozyme frequencies in the two orchards were similar in that alleles most frequent in one were generally most frequent in the other. This was not unexpected because wood specific gravity was the only trait by which the orchard clones were differentially selected. However, important allelic differences occurred, most notably the presence of alleles in one orchard not found in the other. Many of these alleles were also unique to individual clones and could be used to determine how specific clones contribute to the seed crop and how pollen is distributed within and between orchards.

By observing the frequency of unique alleles in the pollen of clones carrying them, we estimated the proportion of selfed progeny to be only 1.2%, based on 513 seeds from five clones. Apparently,

selfing has only minor influence on the genetic makeup of the seed crops in these orchards.

6. Adams, W.T. and R.J. Joly. 1980

Genetics of allozyme variants in loblolly pine. *J. Hered.* 71: 33-40.

It was demonstrated that allozyme variants in 10 enzyme systems are encoded by at least 17 loci, using megagametophytes and embryos of wind-pollinated and control-crossed families of loblolly pine (*Pinus taeda* L.) seed from seed orchard clones. The segregation of allozymes in megagametophytes of heterozygous clones reveals that the single zone of activity on gels stained for glutamate dehydrogenase (GDH) and 6-phosphogluconate dehydrogenase (6PGD) is each controlled by a single locus. The segregation of allozymes in two zones of activity for leucine aminopeptidase (LAP), phosphoglucose isomerase (PGI), phosphoglucumutase (PGM), and glutamateoxaloacetate transaminase (GOT) shows that two loci control each of these enzymes. A third locus, coding GOT variants, seems to be active only in embryos. Acid phosphatase (AP) and malate dehydrogenase (MDH) reveal two zones of activity. In the lower zone of both of these enzymes, single-banded variants occur in megagametophytes, and allozyme segregation supports single-locus control for each. The upper zone of AP, segregating for both single- and double-banded variants, may also be controlled by a single locus. The upper zone of MDH, as well as the single zones of activity observed for ACO and IDH were each invariant for a single band, but indirect evidence inferred from studies in the closely related pitch pine suggests single-locus control for each. In eight loci where embryo band patterns could be interpreted, the same locus was found to code allozymes in embryos and megagametophytes. However, embryo expression in MDH and GOT demonstrate that a gene may not necessarily be detectable in both tissues. Segregation distortions were observed for three heterozygous combinations, and in each case, the deficiency of a particular allele was consistent over several parents.

7. Adams, W.T. and R.J. Joly. 1980.

Linkage relationships among twelve allozyme loci in loblolly pine. *J. Hered.* 17: 199-202.

We investigated linkage relationships among 12 allozyme loci by testing the independence of single-locus segregations in megagametophyte (1n) tissue of seeds from mother trees heterozygous at two or more loci. Forty-nine of the 66 possible two-locus combinations were tested. Segregation of 45 pairs conforms to the hypothesis of independence between loci. Of the remaining four pairs, three seem weakly linked, with estimated recombination fractions ($\hat{P}$) greater than 0.4; the fourth pair (PG12:GOT1) is tightly linked ($\hat{P} = 0.024 \pm 0.007$). Loci coding allozymes with band patterns similar to those of PG12 and GOT1 have also been found to be tightly linked in at least two other conifer species.

8. Allendorf, F.W.; Knudsen, K.L. and Blake G.M. 1982.

Frequencies of null alleles at enzyme loci in natural populations of ponderosa and red pine. *Genetics*, 100: 497-504.

Pinus ponderosa and *P. resinosa* population samples have mean frequencies of enzymatically inactive alleles of 0.0031 and 0.0028 at 29 and 27 enzyme loci, respectively. Such alleles are rare and are apparently maintained by selection-mutation balance. Ponderosa pine have much higher amounts of allozymic and polygenic phenotypic variation than red pine, yet both species have similar frequencies of null alleles. Thus, null alleles apparently do not contribute to polygenic variation, as has been suggested. The concordance between allozymic and polygenic variation adds support to the view that allozyme studies may be valuable in predicting the relative amount of polygenic variation.

9. Bab, Y.K. 1976.

Genetics studies of natural populations of *Pinus densiflora*. I. Mode of inheritance of peroxidase isoenzymes. *Korean J. of Breeding*, 8: 41-47. [Plant Breeding Abstracts 47, 10833].

In an electrophoretic study of four peroxidase isozyme loci in a diallel cross involving 17 *P. densiflora* trees, two alleles, one active and one inactive, were found at each locus. Px_2 K regulates the gene pair Rr. Px_3 M and Px_4 N were found to be on the same linkage group.

10. Bartels, H. 1971.

Genetic control of multiple esterases from needles and macro-gametophytes of *Picea abies*. *Planta*, 99: 283-289.

The patterns of multiple esterases from needles and macrogametophytes of *Picea abies* were examined by polyacrylamide-gel disc-electrophoresis. There are three patterns: (1) two slow-migrating bands, (2) three fast-migrating bands, and (3) a combination of (1) and (2). Re-electrophoresis of the individual bands and genetic analysis demonstrated the existence of two distinct isozymes controlled by two alleles at one locus. In a native population the esterase patterns were in a Hardy-Weinberg equilibrium. In the offspring of crosses between clonal grafts which represent individual trees with different esterase patterns, the patterns segregated in Mendelian ratios. In the haploid macrogametophytes of individual trees they also segregated as expected, i.e., in a 1:1 ratio. This latter fact allows a genetic analysis of individual trees without crossing experiments by investigation of the isozyme patterns only.

11. Bartels, H. 1971.

Isoenzymes and their significance for forest tree breeding and genetics. *Allg. Forstzeit-schr.* 3: 50-52. [Forestry Abstracts 32, 5462].

A general discussion of the basis of the use of enzyme gel electrophoresis in genetics research. This is illustrated by patterns of esterases in *Pinus sylvestris* needles from different clones, showing the use of the method for identification; similar patterns of progeny and parents in *Picea abies* needles indicate that enzymes are suitable gene markers and may be used to elucidate gene structures.

12. Bergmann, F. 1972.

Experiments on possibilities for genetic certification of forest seed. In: Proceedings of the Joint Symposia for Forest Tree Breeding of Genetics Subject Group, IUFRO, and Section 5, Forest Trees. SABRAO. Tokyo, Japan, Government Forest Experiment Station of Japan. A-6(V), 1.5.

Since the statement of the provenance of forest seed which is very important for forestry has frequently been lacking the necessary assurance, numerous experimental approaches for verifying the genetic certificate were introduced. These physiological tests are not always modest and have not often permitted precise results.

A possibility for a rapid and reliable genetic certification of forest seed is based on a method of determining gene frequencies with the aid of isozyme polymorphisms identified in the haploid endosperm of seeds from gymnosperms. In a first attempt a clear discrimination of seed samples from three provenances could be demonstrated by specific gene frequency distributions which resulted from the combination of 5 isozyme loci.

13. Bergmann, F. 1973.

Genetic studies in *Picea abies* with the aid of isoenzyme identification. II. Genetic control of esterase and leucine aminopeptidase isoenzymes in haploid macrogametophytes of dormant seeds. Theor. Appl. Genet. 43: 222-225.

Using techniques of starch gel zone electrophoresis, a considerable variation among esterase and leucine aminopeptidase isoenzyme patterns was found in the endosperm of dormant seeds of Norway spruce (*Picea abies*). Since the so-called primary endosperm of conifers is only a further developmental stage of the haploid female gametophyte, simple Mendelian segregations can be determined in seeds of individual open-pollinated trees. It was therefore possible to identify some esterase and leucine aminopeptidase loci only with regard to phenotypic frequency distributions without difficult crossing procedures.

14. Bergmann, F. 1973.

Genetic studies in *Picea abies* with the aid of isoenzyme identification. III. Geographical variation at 2 esterase and 2 leucine-aminopeptidase loci in the Swedish spruce populations. Silvae Genet. 22: 63-66.

Geographical variation among 8 provenances of the Swedish spruce population (*Picea abies*) was approached by the analysis of genetic polymorphisms at 2 EST and 2 LAP loci in the endosperm of dormant seeds. The resulting gene frequency distributions indicate high polymorphic patterns within populations but only a minor differentiation between individual populations. Clinal variations from north to south, however, were observed in several cases.

Genetic identity and genetic distance values between all pairs of the 8 provenances calculated for better comparisons of the obtained data show an increasing degree of genetic differentiation in parallelism to increasing distance between populations. All results obtained are not in accord with the neutrality hypothesis concerning most isozyme variants.

15. Bergmann, F. 1974.

Genetic distance between populations. II. Determining the genetic distance between European populations of *Picea abies* on the basis of isozyme gene frequencies. Silvae Genet. 23: 28-32.

The genetic distance between 9 spruce provenances (*Picea abies*) from the north and central European area was determined by the aid of a special measure (d_0) proposed in part I of this investigation. The values obtained from this distance measure were related to the genetic composition of populations based on gene frequencies of 4 isozyme loci. The resulting data indicated that a far higher genetic similarity exists between closely located populations than between geographically widely separated provenances. While the greatest genetic distance was found between Scandinavian and German provenances, different other distance values showed a marked genetic divergence between the Finnish and Swedish

provenances, but only a smaller divergence between the selected German populations. It was discussed whether these regionally distributed differences in genetic affinity may depend on the effectivity of isolating barriers.

16. Bergmann, F. 1974.

The genetics of some isoenzyme systems in spruce endosperm (*Picea abies*). Genetika, 6: 353-360.

Using techniques of starch gel zone-electrophoresis, marked variability was detected in the SAP and LAP isoenzyme patterns found in the haploid endosperm of dry spruce seeds (*Picea abies*). Genetic analyses indicated that each of the polymorphic SAP and LAP zones studied is controlled by a distinct gene locus with several alleles. Linkage data showed that two SAP and two LAP loci segregate independently while two EST loci appear to be located on the same chromosome.

17. Bergmann, F. 1975.

Adaptive acid phosphatase polymorphism in conifer seeds. Silvae Genet. 24: 175-177.

In the endosperm of dormant seeds of Norway spruce and Douglas-fir, a gene locus coding for acid phosphatases (SAP) could be identified, which shows a marked environmental-dependent variation. In both tree species, the SAP-alleles coding for single-band enzymes in the zymogram dominate in colder zones, whereas the alleles coding for double-band enzymes are more frequent in all moderate climate zones of the ranges, respectively. It is assumed that one or more temperature parameters represent the selective factors.

18. Bergmann, F. 1975.

Identification of forest seed origin on the basis of isozyme gene frequencies. Allg. Forst. Jagdztg. 146: 191-195.

The numerous experimental procedures so far introduced for the identification of forest seed origin are not applicable in all cases, and have repeatedly led to doubtful results. Therefore, to characterize exactly any provenance a new method has been developed which only uses data of the population-specific gene-pools. These data consist of gene frequency distributions determined at 6 gene loci by means of isoenzyme polymorphisms. Proving this method with 15 Norway spruce provenances from Sweden, Finland, Poland and Germany it could be shown, that the occurrence and frequency of single isoenzyme genes was capable of revealing a clear differentiation of the provenances in their distributional range. Thus, a sufficient characterization of these provenances was possible resulting subsequently in an identification of unknown seed samples, which has been demonstrated in several cases. The only problem as yet unsolved also for this method concerns the provenances and populations that do not represent a homogeneous and autochthonous material.

19. Bergmann, F. 1978.

The allelic distribution at an acid phosphatase locus in Norway spruce (*Picea abies*) along similar climatic gradients. Theor. Appl. Genet. 52: 57-64.

The allele frequency distribution at a polymorphic acid phosphatase locus (APH-B) was determined in natural populations of Norway spruce (*Picea abies*) from a latitudinal transect in Finland, an altitudinal transect in the Austrian Alps, and from different locations of the Swiss range. The three independent population groups, selected with respect to similar climatic gradients, were studied to detect the forces that cause the geographic variation at the APH-B locus.

In almost all of the populations investigated, four alleles (APH-B₁ — B₄) could be identified at this enzyme locus, however, the alleles B₁ and B₂, as well as B₃ and B₄, show a great similarity according to their phenotypic appearance after electrophoresis as well as to their frequency distributions along the different transects. With the aid of some theoretical considerations and data comparisons,

comparisons, a selective equivalence of the alleles B_1 and B_2 , as well as B_3 and B_4 , could be ascertained, thus reducing the number of alleles that can respond differently to natural selection.

After combining the frequencies of the selectively equivalent alleles, similar clinal variation patterns could be observed along the different geographical transects, whereby the frequency of the allele group APH- B_1/B_2 markedly increases with latitudes in Finland and towards higher elevations in the Alps. Correspondingly, the allele group APH- B_3/B_4 predominates in the southern parts of Finland and in the lowlands and foothills of Austria and Switzerland. It is therefore concluded that natural selection causes the geographic variation pattern at the APH-B locus and that one or several temperature variables function as an at least predominant selective force. Possible relationships between this enzyme polymorphism and other tree characters and the physiological role of acid phosphatases in tree adaptation were discussed.

20. Bergmann, F. and H.R. Gregorius. 1979.

Comparison of the genetic diversities of various populations of Norway spruce (*Picea abies*). In: Proceedings of the Conference on Biochemical Genetics of Forest Trees, Umeå, Sweden, 1978. pp. 99-107.

By means of starch gel electrophoretic surveys, the genic structure (allele frequencies) at seven enzyme loci was determined for 21 natural Norway spruce populations which are located in Sweden, Finland, Poland, Germany, Austria, Switzerland and Rumania. Based on the resulting gene frequency data, the intrapopulational genetic variability (diversity) was specified by several common variability measures (e.g. proportion of polymorphic loci, heterozygosity) as well as by a specific measure of genic diversity developed by GREGORIUS. The latter measure has the characteristic properties of being linear with respect to the number of alleles for uniform frequency distributions and allowing for application to arbitrary numbers of gene loci.

When comparing the genic diversity data among the spruce populations, marked differences could be observed not only between different re-

gions of the natural range but also between different populations of the same region. In some cases, the genic diversity is lower in populations from the borders of a transect (which could be marginal populations) than in populations from the center, which reflects the observation that clinal variation at several loci tends towards extreme allele frequency distributions at the borders of the transect.

21. Bonnet-Masimbert, M. and V. Bikay-Bikay. 1978.

Intraspecific variability of isozymes of glutamate-oxaloacetate-trans-aminase in *Pinus nigra* Arnold. of value in the taxonomy of the species. *Silvae Genet.* 27: 17-79.

Pinus nigra Arn., a collective species with disjoint distribution possesses a lot of taxonomic problems. Generally four subspecies are recognized: *laricio*, *clusiana*, *nigricans* and *pallasiana*. Several studies of morphology and monoterpenes composition allow only a partial solution to the problem of precise distinction between subspecies. In particular, it is difficult to separate *nigricans* from *pallasiana*.

After starch gel electrophoresis of dormant seed haploid endosperms belonging to 163 half-sib progenies among the 4 subspecies, we identified 4 loci (with 2, 3, 6 and 3 alleles respectively) in glutamate oxaloacetate transaminase isozymes (GOT). B and C, the middle loci, are most interesting. We compared allele frequencies for 40 provenances distributed among subspecies. Analysis of these results by 3 different methods genetic distances, factorial correspondence analysis (i.e. reciprocal averaging) dendrograms led us to conclude that: 1 — *laricio* and *pallasiana* are more homogeneous in their GOT composition than *clusiana* and *nigricans*. 2 — Composition of isozymes does not reveal any clinal variation probably as a result of genetic drift. 3 — *Clusiana* has characteristic banding patterns (C_0 , C_4 , C_8). 4 — We observed few differences between *laricio* from corsica and calabria. 5 — They are closer to *pallasiana* than the other subspecies. 6 — Above all, the C_3 allele, which is abundant for *nigricans* and missing for *pallasiana*, offers a good way for distinguishing between the two subspecies.

22. Brown, A.H.D.; A.C. Matheson and K.G. Eldridge. 1975.

Estimation of the mating system of *Eucalyptus obliqua* L'Hérit. by using allozyme polymorphisms. Aust. J. Bot. 23: 931-949.

Seeds from four populations of *E. obliqua* were assayed for their allozyme genotype at three loci (alcohol dehydrogenase, malate dehydrogenase and acid phosphatase) as members of open-pollinated arrays to obtain quantitative estimates of three mating system parameters. These were (i) the proportion of seed derived from self-pollination as distinct from the proportion randomly outcrossed, (ii) the allele frequency in the pollen, and (iii) the within-population heterogeneity for the frequency of detectable outcrosses. The rate of selfing varied between loci and populations, but overall suggested that up to 24% of viable seed may derive from self-fertilization. This level of partial self-fertilization, together with local variation in the mating system, yielded an observed average inbreeding coefficient (Wright's fixation index) of 0.123 for these loci. In addition, the populations showed substantial differences in gene frequency at each locus. The statistical techniques appropriate for the analysis of open-pollinated progeny arrays are given in an appendix.

23. Brown, A.H.D. and G.F. Moran. 1979.

Isozymes and the genetic resources of forest trees. In: Proceedings of Symposium on Isozymes of North American Forest Trees and Insects, July 23, 1979, Berkeley, Calif. Gen. Tech. Rep. PSW-48. Pacific Southwest Forest and Range Exp. Sta., Forest Serv., U.S. Dep. Agric., Berkeley, Calif., pp 1-10.

Genetic data are an essential prerequisite for analyzing the genetic structure of tree populations. The isozyme technique is the best currently available method for obtaining such data. Despite several shortcomings, isozyme data directly evaluate the genetic resources of forest trees, and can thus be used to monitor and manipulate these resources. For example, preliminary isozyme data indicate that domestication of cultivated species has generally reduced variation within populations. Geographic differences among natural

populations are less evident in trees than in herbaceous plants, but need to be considered in sampling and conservation strategies. In dealing with remnant population size (N_r) and distribution, management programs should recognize that the half-life of heterozygosity is about $1.4 N_r$ generations, whereas the number of trees required to retain half the current alleles after a bottleneck is about the square root of the original population size.

24. Cheliak, W.M. and B.P. Dancik. 1982.

Genic diversity of natural populations of a clone-forming tree *Populus tremuloides*. Can. J. Genet. Cytol. 24: 611-616.

Effects of asexual reproduction as a primary reproductive strategy on population structure and levels of variability were investigated electrophoretically in natural populations of a woody plant species, trembling aspen (*Populus tremuloides* Michx.), from Alberta. As expected, levels of genic diversity, 42%, and proportion of polymorphic loci, 92%, averaged over all clones are considerably greater than those reported for comparable samples of sexually reproducing plant and animal species. These measures of genic variability of a primarily asexual plant species are similar to those reported for asexual species of insects, fish and bacteria. In addition, each of the 222 clones was electrophoretically unique. Since neutral theory would predict each individual clone to be heterozygous for a unique mutation at each gene locus at equilibrium, these results can be interpreted in a number of ways: (i) insufficient time to reach equilibrium, (ii) inability of electrophoresis to detect all variation at a locus, (iii) periodic establishment of sexually derived propagules in the population, and (iv) selection for similar genotypes at each location or against mutations at particular gene loci. Re-invasion of Pleistocene-glaciated areas by trembling aspen likely was by sexual means, with subsequent reproduction being primarily asexual.

25. Cheliak, W.M. and J.A. Pitel. 1984

Genetic control of allozyme variants in mature tissues of white spruce trees. J. Hered. 75: 34-40.

A method for extracting enzymes from mature tissues of coniferous species was applied to 280 progeny from a controlled mating scheme involving five white spruce (*Picea glauca* (Moench) Voss) trees. Formal analysis of the genetic control of allozyme polymorphisms at six loci was made with this material. Numerous other loci lacking variation among parental trees also were investigated. Genotypic expression of isozyme loci in two types of diploid tissues and one source of haploid tissue from these plants was consistent for those enzyme loci that were common to all tissue types.

26. Chung, M.-S. 1981.

Biochemical methods for determining population structure and mating system in *Pinus sylvestris* L. Acta For. Fenn. 173, 28 pp.

Studies on Finnish Scots pine plus tree clones by monoterpene and isozyme analyses have been undertaken to further investigate mating system, population structure and pollination. Six allozyme systems (8 GOT, 1 GDH and 2 LAP) were properly analysed on the basis of segregation. Monoterpenes were analysed from needle material and segregation in high and low 3-carene content was found to depend on two alleles, C and c.

Thus six allozyme systems and one monoterpene system were used as markers in this study. It was found:

- That the northern clonal group maintains as much genetic variation as the central or southern clonal groups.
- That the conditional probability of self-fertilization in about 20-year old clones estimated by multilocus model was 14.1%, of which 8.0% originate from matings between trees that carry the same alleles to one of the maternal parents at some loci and 6% through self-fertilization.
- That there was no prominent difference in allele frequencies of male gametes that pollinated the very early or very late flowering clones.

- That the northern clonal group has higher and lower frequencies of alleles GOT B₂ and B₃ respectively than those of the southern clonal group.
- That artificial plus tree selection, particularly in northern Finland, appears to favor heterozygous genotypes for the alleles that control 3-carene content in Scots pine.

27. Clarkson, R.B.; F.H. Huang; F.C. Cech and L.A. Gingerich. 1974.

Variability of soluble seed proteins, peroxidase and leucine aminopeptidase, in populations of *Juglans nigra*. Biochem. Syst. Ecol. 2: 59-66. [Forestry Abstracts 37, 2134].

Reports on investigation of the variability of proteins and iso-enzymes in seeds from 94 trees representing 17 populations throughout the geographic range of *J. nigra*. Results indicated that variations between populations were always greater than variations within them.

28. Conkle, M.T. 1971.

Inheritance of alcohol dehydrogenase and leucine aminopeptidase isozymes in knobcone pine. For. Sci. 17: 190-194.

A new technique for analyzing the genetic structure of trees is available. Enzymes from germinating embryos were resolved by starch gel electrophoresis. Three polymorphic loci were identified in related families of knobcone pine (*Pinus attenuata* Lemm). Progenies in 16 control-pollinated families from 12 parent trees were tested. Isozymes for one alcohol dehydrogenase (ADH) locus and for two leucine aminopeptidase (LAP) loci segregated according to the laws of diploid inheritance, with two alleles per locus. Segregation in the progeny of a parent tree heterozygous for one ADH and one LAP locus showed that two loci are unlinked.

29. Conkle, M.T. 1971.

Isozyme specificity during germination and early growth of knobcone pine. *For. Sci.* 17: 494-498.

Five enzyme classes from 11 developmental stages of germinating embryos were separated by starch gel electrophoresis. Alcohol dehydrogenase isozymes found in embryos of dry seed were most active at the time of radicle emergence; activity decreased thereafter, fading below the level of detection when seed coats were shed. Peroxidase isozymes were absent and esterase isozymes were nearly absent from stratified seed, but both increased steadily in number and stainability from time of radicle emergence through epicotyl elongation. Leucine aminopeptidase and catalase isozymes were detectable in all growth stages. In later stages, esterases and leucine aminopeptidases were found primarily in seedling tops, and many catalase and peroxidase isozymes differed between roots and tops. Both the developmental state of the plant and the tissue assayed determine the presence of specific isozymes.

30. Conkle, M.T. 1972.

Analyzing genetic diversity in conifers — isozyme resolution by starch gel electrophoresis. USDA Forest Serv. Res. Note PSW-264. Pacific Southwest Forest and Range Exp. Sta., Berkeley, Calif. 5 p.

Enzymes in forest tree materials can be resolved by starch gel electrophoresis. A gel slab is prepared in a mold assembled from glass and plastic. Wicks containing an aqueous extract of macerated plant material are inserted in the gel and processed. The gel is sliced, stained, examined, and photographed. Isozyme bands produced by differential migration of enzymes indicate genetic segregation and recombination. This technique is familiar in other uses, but the procedure described here is specifically adapted to conifers.

31. Conkle, M.T. 1974.

Enzyme polymorphism in forest trees. *In: Proceedings of the Third North American*

Forest Biology Workshop, 9-12 September 1974, Colorado State University. pp. 95-105.

Until recently, forest biologists had only limited means of resolving differences at the level of individual gene loci. The above examples illustrate the genetic resolving power of the electrophoresis technique. As gene markers, isozymes are useful in describing genetic differentiation within and between taxa and for varifying hybridity. They should also prove useful for monitoring pollen migration, determining the degree of selfing, and testing panmixis. Isozymes offer a means of studying phenotypic response over environmental gradients where genetic material can be identified by specific allelic combinations.

32. Conkle, M.T. 1979.

Amount and distribution of isozyme variation in various conifer species. *In: Proceedings 17th Meeting of the Canadian Tree Improvement Association. Symposium: Tree Improvement in the Boreal Forest: Today and Tomorrow.* pp. 107-117.

Estimation of the relative amount and geographic distribution of genetically controlled variation is a central topic of tree resource management. Biochemical data from the analysis of forest tree enzyme variants provides a direct and precise measure of allele frequencies of tree genes.

The amount of genetic variation in several conifers; Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), loblolly pine (*Pinus taeda* L.), sugar pine (*P. lambertiana* Dougl.), knobcone pine *P. attenuata* Lemm.), lodgepole pine (*P. contorta* Var. *latifolia* Engelm.), and Jeffrey pine (*P. jeffreyi* Grove and Balfour) is assessed by determining the number of alleles per locus and species heterozygosities. Both measures indicate that native conifers are genetically variable and species differ in the kind and amount of variation they contain. Comparisons with herbaceous plants rank conifers among the most variable plants thus far studied.

The distribution of genetic variation measured by analysing stands and geographic samples is an active area of research. Published studies and current unpublished work leads to the conclusion

that geographic trends can be identified that support the hypothesis that subsamples are significantly differentiated with respect to allele frequencies but the major amount of variation resides within the subsamples. Studies using isozyme data are providing data on breeding systems and comparisons of life history characteristics are showing consistent patterns which characterize the genetic strategies of forest trees.

33. Conkle, M.T. 1979.

Isozyme variation and linkage in six conifer species. *In*: Proceedings of Symposium on Isozymes of North American Forest Trees and Insects, July 27, 1979, Berkeley, Calif. Gen. Tech. Rep. PSW-48. Pacific Southwest Forest and Range Exp. Sta., Forest Serv., U.S. Dep. Agric., Berkeley, Calif., pp 11-17.

Isozymes of female gametophyte tissue were analyzed for allelic variation in knobcone, lodgepole, loblolly, Jeffrey, and sugar pines and in Douglas-fir. Linkage was studied in five pines. The average number of alleles and average heterozygosity per enzyme locus were estimated. Knobcone pine ranked lowest among the six species in number of alleles and average heterozygosity; loblolly pine and Douglas-fir ranked highest. Numerous linkages were identified, and one chromosome segment contained between one-fourth and one-third of all loci examined. Linked loci had a consistent gene order in the different pine species. Linkage relationships support the concept that similar isozymes represent the same loci in each species. The consistency with which loci map in the same order and with similar map distances in species from different subsections of the genus, further suggests that evolution of the pines is not associated with major chromosome rearrangements.

34. Conkle, M.T. and W.T. Adams. 1977.

Use of isoenzyme techniques in forest genetics research. *In*: Proceedings of the 14th Southern Forest Tree Improvement Conference, Gainesville, Florida. June 14-16, 1977. pp. 219-226.

Genetic variation among loblolly pine (*Pinus taeda* L.) samples from a natural stand and among clones in seed orchards was analyzed using simply inherited isozyme markers. Alleles for eleven enzyme loci were found useful for genotyping trees in a natural stand in North Carolina. The pines were highly variable with as many as seven alleles per isozyme gene. On the average, close to 30 percent of the loci per tree were heterozygous. Similar levels of variability and heterozygosity were found among the clones in two seed orchards. Such variability makes it possible to uniquely identify most, if not all, of the clones in a seed orchard. Additional genes and other southern pine species are suited for similar analyses. A study with 15 different enzyme systems and samples from six species; loblolly, shortleaf, slash, pond, longleaf, and Virginia pines, suggested that as many as 26 different loci may be available for analysis and most loci appear to be similar for these species.

35. Conkle, M.T.; P.D. Hodgskiss; L.B. Nunally and S.C. Hunter. 1982

Starch gel electrophoresis of conifer seeds: a laboratory manual. Gen. Tech. Rep. PSW-64. Pacific Southwest Forest and Range Exp. Sta., Forest Serv., U.S. Dep. Agric., Berkeley, Calif. 18 p.

This manual describes fast, low-cost biochemical procedures for separating enzymes representing numerous genes of forest trees. During electrophoresis the mixture of enzymes from a megagametophyte or embryo of a germinated seed separates in a gel. Specific stains applied to gel slices locate each enzyme. These procedures expand on those developed for crops research. They provide a means for forest geneticists to get urgently needed information on the amount and geographic distribution of genetic variation in conifers for evaluating species relationships, for protecting rare natural populations, and for deciding on breeding programs for commercial species. Electrophoresis of conifers is an alternative to long-term, high-cost field trials.

36. Conkle, M.T. and J.J. Tobolski. 1981.

Isozymes of linked loci segregate normally in seeds of an Austrian $\times$ Japanese red pine hybrid. In: Proceedings of the Second North Central Tree Improvement Conference, Lincoln, Nebraska, pp. 195-200.

An F₁ *Pinus nigra* and *P. densiflora* hybrid was polymorphic for seven enzyme loci. Data from haploid female gametophytes of the hybrid established that four of the seven loci were linked in one block. Three loci segregated independently: acid phosphatase 1, glutamic oxaloacetic transaminase 3, and glucose-6-phosphate dehydrogenase 1/2. The linked loci were alcohol dehydrogenase 1, alcohol dehydrogenase 2, glucose-6-phosphate dehydrogenase 2, and leucine aminopeptidase 2, arranged in that order. The recombination percentages between linked loci were 9, 12, and 14, respectively. The order and recombination percentages of the linked loci were similar to values reported for *P. sylvestris*, another pine in the subsection *Sylvestris* and other species in *Pinus*. The hybrid provides a means of enriching genetic variability by producing allelic combinations that do not exist in natural populations.

37. Copes, D.L. 1975.

Isoenzyme study of dwarf and normal Douglas-fir trees. Bot. Gaz. 136: 347-352.

Isoenzymes of 13 yewlike dwarfs, 13 twisted-needle dwarfs, and 13 normal Douglas-fir trees were compared for seven enzymes. Dwarfing altered the trees, and many zymograms were significantly different from zymograms of normal trees. Dwarfs were characterized by one or more of the following traits: the presence of some isoenzymes not found in normal trees, the lack of some isoenzymes that were present in all normal trees, and the darker staining of some isoenzymes than of the same isoenzymes in normal trees. These differences were in addition to the usual differences between trees and between tissues or organs found in normal Douglas-fir populations. No one isoenzyme typified all yewlike or all twisted-needle dwarfs.

38. Copes, D.L. 1978.

Isoenzyme activities differ in compatible and incompatible Douglas-fir graft unions. For. Sci. 24: 297-303.

Unusually dark-stained peroxidase bands at R_m (relative mobility) 0.77 to 0.80 and esterase bands at R_m 0.50 to 0.56 were found in tissues of incompatible Douglas-fir graft unions. Ninety- to 100-percent accuracy in identifying incompatible grafts was achieved with union tissues 10 to 15 months after grafting (February to May). A practical graft testing program is possible using electrophoretic methods for detecting changes in stain intensity in bands from incompatible graft unions. Enzyme activity of bands with other R_m values was not correlated, or was only weakly correlated, with graft incompatibility. Enzyme similarity of stock and scion tissues could not be used to determine potential compatibility of stock-scion combinations before grafting. No correlation with graft incompatibility was found with enzyme activity or the presence or absence of isoenzyme bands of catalase, acid phosphatase, or leucine aminopeptidase.

39. Copes, D.L. 1979.

A genetic analysis of aminopeptidase and peroxidase isoenzymes in Douglas-fir parent trees and seedling progeny. Can. J. For. Res. 9: 189-192.

The genetic control of isoenzymes found in bark and vegetative buds was determined for one peroxidase and one aminopeptidase locus. The segregation of alleles within full-sib progeny of a 6 $\times$ 6 tree diallel fitted expected Mendelian ratios. Eight peroxidase isoenzymes existed, but only those with relative mobilities of 0.44 and 0.49 showed allelism. Several of the other six peroxidase bands gave a false impression of segregation.

40. Copes, D.L. 1981.

Isoenzyme uniformity in western red cedar seedlings from Oregon and Washington. Can. J. For. Res. 11: 451-453.

Isoenzymes of newly germinated western red cedar seedlings (*Thuja plicata* Donn ex D. Don) from western Oregon and eastern and western Washington lacked variation in band patterns of nine enzymes. The isoenzyme results indicate that western red cedar populations contain very little genetic polymorphism. Management of the species will likely be more productive if concentrated on cultural practices rather than on intensive genetic improvement.

41. Copes, D.L. and R.C. Beckwith. 1977.

Isoenzyme identification of *Picea glauca*, *P. sitchensis*, *P. lutzii* populations. Bot. Gaz. 138: 512-521.

Electrophoretic techniques were used to identify stands of pure Sitka spruce *Picea sitchensis* (Bong.) Carr. and pure white spruce *P. glauca* (Moench) Voss and spruce stands in which introgressive hybridization between the white and Sitka spruce had occurred. Thirteen heteromorphous isoenzymes of LAP, GDH, and TO were the criteria for stand identification. Estimates of likeness or similarity between seed-source areas were made from D determinations. Introgressed hybrid stands had isoenzyme frequencies that were intermediate between the two pure species, but the seedlings were somewhat more like white spruce than like Sitka spruce. Much of the west side of the Kenai Peninsula appeared to be a hybrid swarm area, with stands containing both Sitka and white spruce genes. The presence of white spruce genes in Sitka spruce populations was most easily detected by the presence of TO activity at Rm. 52. White spruce showed activity at that position in 79% of its germinants; only 1% of the pure Sitka spruce germinants had similar activity. Isoenzyme variation between stands of pure Sitka spruce was less variable than that between interior white spruce stands (mean distinction values were 0.11 for Sitka and 0.32 for white spruce). Cluster analysis showed all six pure Sitka spruce populations to be similar at .93, whereas pure white spruce populations were not similar until .69.

42. Dancik, B.P. and Yeh, F.C. 1983.

Allozyme variability and evolution of lodgepole pine (*Pinus contorta* var. *latifolia*) and jack pine (*P. banksiana*) in Alberta. Can. J. Genet. Cytol. 25: 57-64.

Genetic variability in five lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) and three jack pine (*P. banksiana* Lamb.) populations from Alberta was analyzed electrophoretically for 14 enzymes presumably representing 21 structural loci. Single populations of lodgepole pine were, on the average, polymorphic and heterozygous at 51.4 and 18.4% of their loci, respectively, and had 2.5 alleles per locus. In contrast, jack pine populations were less variable genetically; the average number of alleles per locus was 2.1, and 46 and 11.5% of their loci were polymorphic and heterozygous, respectively. Analysis of *F* statistics showed a 2.5 and 9.7% deficiency of heterozygotes relative to Hardy-Weinberg expectations for lodgepole pine and jack pine populations, respectively. Only 2% of the observed genetic variability in each species appeared to be interpopulation, the remainder was due to differences among individuals within populations, in agreement with results from other conifers. Average genetic distance between the two species was 20 times greater (0.097) than between populations within species (0.005). In general, the enzyme data were concordant with earlier hypotheses that the two species evolved from a common progenitor.

43. Djupsund, B.M. 1978.

Electrophoretic separation techniques on horizontal thin layers. In: Proceedings of the Conference on Biochemical Genetics of Forest Trees, Umea, Sweden, 1978. pp. 16-24.

Thin layer electrophoresis in horizontal agarose gels is reviewed. The methodological pros and cons are summarized and preliminary application for analysis of proteinaceous extracts from *Pinus sylvestris* L. is reported.

For more accurate separation requirements the thin layer gel electrofocusing has recently become a most popular fractionation and charac-

terization technique. It combines superior resolution with additional information concerning the isoelectric points of the focused proteins. In a preparative mode, isoelectric focusing in granulated horizontal thin layers has proved to be useful for biochemical purification and isolation purposes.

44. Dukharev, V.A. 1978.

Composition of the esterases in *Pinus silvestris* in uneven-aged stands. Lesovedenie, 6: 45-50. [Forestry Abstracts 40, 4560].

A study was made of the frequency of occurrence of the alleles of isozymes of 4 enzymes in needles of trees of different ages in two 2-storeyed stands of *Pinus sylvestris*, one in the Moscow region and the other in the Mari ASSR. The results are tabulated. The frequency of occurrence of the alleles by the four loci was identical in trees of the first and second storeys in the two stands. The ratio of esterase genotypes deviated more from the Hardy — Weinberg law in the first storey than in the second. The number of heterozygotes increased, and the number of homozygotes decreased, with increasing age of the trees.

45. Dukharev, V.A. 1978.

Investigation of esterase polymorphism in the population of *Pinus silvestris* L. Genetika, 14: 1789-1793.

Four regions (A, B, C, D) of the esterase activity, having a different sensitivity to inhibitors, were found in seed endosperm and needles of pine. They were determined as arylesterase (A), carboxylesterase (B), acetylesterase (C), carboanhydrase (D). A, B, C and D regions in homozygous state, like endosperm tissue, have single bands of the enzyme activity in diploid needles tissue, while in heterozygous state the regions B, C and D have a 2-band pattern. The A region in heterozygous state has 3 bands of the enzyme activity.

46. Durzan, D.J. 1966.

Disc electrophoresis of soluble protein in the female gametophyte and embryo of conifer seed. Can. J. Bot. 44: 359-361 [A note].

47. Eckert, R.T.; R.J. Joly and D.B. Neal. 1981.

Genetics of isozyme variants and linkage relationships among allozyme loci in 35 eastern white pine clones. Can. J. For. Res. 11: 573-579.

Megagametophyte tissue from seeds of 35 clones of *Pinus strobus* L. were subjected to horizontal starch gel electrophoresis. The resulting gels were tested for activity of 10 enzyme systems. Isozymes observed were under control of at least 17 loci. Direct evidence, based on segregation of allozymes in megagametophytes of heterozygous clones, is presented for nine loci in five systems (LAP, GPI, GOT, PGM, and AP). Three polymorphic loci were detected for LAP. No genetic variation was detected for IDH, ADH, GLY-3-PD, GDH, or 6 PDG.

Five pairs of linked loci were detected by chi-square analyses of joint segregation. Twenty-five of 36 possible two-locus combinations among 18 clones were evaluated. Recombination estimates suggest relatively strong linkage between LAP1-LAP2 and GPI2-GOT1; weaker linkage between LAP2-GPI2 (based on one clone only); and weak linkage between LAP2-API and GOT3-API.

48. EL-Kassaby, Y.A. 1981.

Genetic interpretation of malate dehydrogenase isozymes in some conifer species. J. Hered. 72: 451-452.

Among conifer species, the resolution of malate dehydrogenase (MDH) band patterns was greatest in Douglas-fir in which there was evidence of four loci, two of which regularly form a heterodimeric product. MDH in lodgepole pine, white spruce, and Sitka spruce also appear to be under control of four loci, while MDH in the true firs,

white and grand fir, and western red-cedar appear to be controlled by only three loci.

49. EL-Kassaby, Y.A. 1982.

Association between allozyme genotypes and quantitative traits in Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco]. Genetics, 101: 103-115.

Effects of maternal genotypes for eight enzyme loci on seven quantitative traits in 41 half-sib families were determined. Heritability estimates for the quantitative traits were highly significant and ranged from 0.57 to 0.86. Only six of 112 comparisons (56 comparisons for each of the genotypes and 46 for the homozygous versus heterozygous contrasts) were statistically significant ($P < 0.05$). None of these significant comparisons could be associated with either a particular allele or a particular genotype. No heterotic effects were observed when the homozygous genotypes were compared with the heterozygous ones. Because the quantitative traits are highly correlated, a discriminant function analysis was performed to obtain the two most important canonical variates that accounted for 75% of the variance in the quantitative traits. There were no significant associations among enzyme loci and the two canonical variates. I conclude that, in this study, enzyme genotypic differences do not influence the quantitative traits to a major extent.

50. EL-Kassaby, Y.A. and O. Sziklai. 1982.

Genetic variation of allozyme and quantitative traits in a selected Douglas-fir [*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco] population. For. Ecol. Manage. 4: 115-126.

The genetic variation at 27 allozyme loci and seven different seedling traits were studied in a coastal stand of Douglas-fir [*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco] with respect to different elevation classes. The average proportion of polymorphic loci per elevation class was 0.54 ± 0.06 and the average proportion of heterozygous loci per individual was 0.17 ± 0.02 . The isozyme data showed that the amount of genetic

polymorphism varied considerably between loci and the amount and pattern of genetic variation for most loci were similar among the different elevational classes although some loci had large differences among elevational classes.

Seven % of the total genic diversity was attributed to interelevational differentiation while the remaining 93% resided within the different elevational classes.

Good agreement occurred between the isozyme data and quantitative information obtained from half-sib families of the same trees used for the isozyme analysis. The analyses of variance for the studied traits gave the same general trend and variation within the different elevational classes varied between 94 and 100%.

In general, both the quantitative and the isozyme studies showed that the majority of variation resided within and not among the different elevation classes. Using these two different approaches in assessing variation in natural population is recommended.

51. EL-Kassaby, Y.A. and O. Sziklai. 1983.

Effect of sample size on the precision of the estimate of allozyme frequencies in a natural stand of Douglas-fir. Egypt. J. Genet. Cytol. 12: 345-360.

This study describes the effect of sample size on the estimates of allelic frequencies and their precision. A total of 42 trees from a natural stand of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] were genotyped for 27 allozyme loci using standard horizontal starch gel electrophoresis. Diallelic data were generated for the 18 polymorphic loci studied. These loci were grouped into three arbitrary categories according to the frequency of their rare allele. A random number generator program was used to select random samples with different sizes varying between 5 and 42. For each sample the allelic frequencies and their confidence intervals were estimated and plotted against their corresponding sample size. The observed results demonstrated a sharp fluctuation in the allelic frequencies' estimates and a wide range of confidence intervals when a small sample size was used. As sample size

increased, a stable condition for the allelic frequencies together with narrow confidence intervals were observed. It is concluded that a sample size between 40 to 60 trees is adequate to obtain reliable estimates of populations' allelic frequencies.

52. EL-Kassaby, Y.A.; Sziklai, O. and F.C. Yeh. 1982.

Linkage relationships among 19 polymorphic allozyme loci in coastal Douglas-fir *Pseudotsuga menziesii* var. *menziesii*. Can J. Genet. Cytol. 24: 101-108.

Linkage relationships among 19 different polymorphic loci in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco) were studied. A total of 115 of 171 possible two-locus combinations were tested. Out of the 115 combinations, a total of 106 conform to the hypothesis of independence between loci indicating that no linkage exists between these pairs. Of the remaining nine pairs, two were tightly linked with recombination frequencies of 0.02 (*Aat2:Pgi2*) and 0.22 (*Aat3:Sod*). The remaining were loosely linked, with recombination frequencies varying between 0.33 and 0.42. Two of these nine linked pairs have been detected in other conifer species. It was not possible to study three-point linkages due to the lack of appropriate combinations. To our knowledge, these results provide the first demonstration of genetic linkage in Douglas-fir.

53. EL-Kassaby, Y.A.; Yeh, F.C. and O. Sziklai. 1981.

Estimation of the outcrossing rate of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] using allozyme polymorphisms. Silvae Genet. 30: 182-184.

The haploid megagametophyte and corresponding diploid embryo tissues from 36 seed trees from a natural stand of coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were assayed for electrophoretically demonstrable variation in proteins at four polymorphic loci (glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, phosphoglucosmutase, and 6-

phosphogluconic dehydrogenase). The probability of detecting genotypic arrays of progeny from seed trees of known genotypes was used as an estimator of outcrossing rate (t). The level of outcrossing ($t = 0.9$) yielded an average inbreeding coefficient (F) of 0.05 for these loci. It is not known whether this level of inbreeding is common in coastal Douglas-fir. However, if such inbreeding levels are common, some of the improvement in performance seen in progenies from wind-pollinated controls, may result from seed orchards over progenies from the break up of the family structure of the stands.

54. EL-Kassaby; Y.A., Yeh, F.C. and O. Sziklai. 1982.

Inheritance of allozyme variants in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Can. J. Genet. Cytol. 24: 325-335.

The inheritance of 27 loci from 18 enzyme systems was investigated using both megagametophyte and embryo tissues of open pollinated seed collected from a natural stand of coastal Douglas fir [*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco]. Four enzyme systems — glutamate dehydrogenase (GDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), mannose-6-phosphate isomerase (MPI), and peptidase (PEP) — appeared to be monomorphic and the remaining 14 systems — acid phosphatase (APH), aconitase (ACO), aspartate aminotransferase (AAT), diaphorase (DIA), esterase (EST), glucose-6-phosphate dehydrogenase (G6P), hexosaminidase (HA), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucose isomerase (PGI), phosphoglucosmutase (PGM), 6-phosphogluconic dehydrogenase (6-PGD), and superoxide dismutase (SOD) showed polymorphism. Each enzyme system was discussed with respect to its multilocus organization, subunit (quaternary) structure, and allelic variation.

55. Epperson, B.K. and R.W. Allard. 1984.

Allozyme analysis of the mating system in lodgepole pine populations. J. Hered. 75: 212-214.

The mating system was investigated in two natural populations of lodgepole pine (*Pinus contorta* spp. *latifolia*). Single- and multilocus estimates of outcrossing rates were not statistically different from 1.0, indicating that both populations were nearly 100 percent outcrossing. Wright's fixation index was near zero in both the mature trees and their seed. This result and the homogeneity of the single-locus estimates suggest that the assumptions made in the mixed mating system model were not substantially violated in the population studied. This is in contrast to other studies of the mating system in outcrossing species in which significant departures from random mating have been observed.

56. Feret, P.P. 1971.

Isoenzyme variation in *Picea glauca* (Moench) Voss seedlings. *Silvae Genet.* 20: 46-50.

Using techniques of polyacrylamide disc gel electrophoresis, peroxidase and esterase isoenzyme variation was studied in individual seedlings of half sib, F_1 and S_1 *P. glauca* families. A total of ten peroxidases and four esterases were analyzed. Seedling isoenzyme patterns were correlated with parental isoenzyme patterns but no specific genetic hypothesis could be formulated to explain the isoenzyme variation observed. The existence of genic or environmental regulator elements affecting isoenzyme expression was discussed.

57. Feret, P.P. 1972.

Peroxidase isoenzyme variation in interspecific elm hybrids. *Can. J. For. Res.* 2: 264-270.

Analysis of peroxidase isoenzymes in interspecific elm hybrids indicated that a close correlation exists between parental isoenzymes and those of the progeny. In some crosses, isoenzymes undergo Mendelian segregation while in others segregation distortion is evident. Mechanisms proposed to account for the disturbed segregation ratios and unexpected phenotypes include crossing over between peroxidase loci, accumulation

of lethal genetic deficiencies, and dominance of some peroxidase genes.

58. Feret, P.P. 1974

Genetic differences among three small stands of *Pinus pungens*. *Theor. Appl. Genet.* 44: 173-177.

Analysis was made of isoenzyme and morphological differences among three small adjacent stands of *Pinus pungens* Lamb. and progeny derived from the stands. Results indicated that although significant differences were observed in measured characteristics among stands, progeny derived from the stands did not differ significantly. Analysis of gene frequencies for two esterase phenotypes indicated that all three stands arose from a single panmictic population. The results of the analysis might be explained by environmental effects on the characteristics measured *in situ*, by genetic drift or by natural selection.

59. Feret, P.P. 1979.

Isozymes for tree improvement. *In: Proceedings of the 26th N.E. For. Tree Improvement Conference, Penn. State Univ., USDA Forest Serv., NE For. Exp. Sta.* pp. 102-120.

In summary, isozyme analysis of trees is making significant contributions to tree improvement. Applications are becoming increasingly frequent and in the future will probably be even more frequent. The full realization of the potential of isozyme analysis will be realized when cooperative research undertaken by silviculturists, physiologists and geneticists is integrated and applied to the basic problems of improving survival, growth and yield.

60. Feret, P.P. and F. Bergmann. 1976.

Gel electrophoresis of proteins and enzymes. *In: Modern Methods in Forest Genetics.* Springer-Verlag, New York (ed. J.P. Miksche). pp. 49-77.

Although the techniques of gel electrophoresis of proteins and enzymes are not new, their use in the study of forest tree genetics is recent. The potential uses of the techniques are considerable, yet by no means are they currently being exploited by forest tree geneticists. Although rudimentary knowledge of genetically caused isoenzyme variability is available for few *Pinus* species, *P. abies*, *Ulmus* spp., most tree genera have not been investigated. Clearly the technique can provide solutions to a number of basic and applied problems in forest tree genetics and tree improvement. In the future, serious application of gel electrophoresis should greatly expand the body of knowledge in forest genetics.

61. Feret, P.P. and R.L. Bryant. 1974.

Genetic differences between American and Chinese *Ailanthus* seedlings. *Silvae Genet.* 23: 144-148.

By use of comparison of regression coefficients, analysis of variance, discriminant analysis and isoenzyme analysis, American *ailanthus* seedlings were found to be distinctly different from seedlings of Chinese seed sources. Over four harvests of seedlings from both countries, eleven of fourteen variables studied showed significant country differences. Thus, under the assumption that representative gene pools were used for experimentation significant alteration of genetic content has occurred in the introduction and naturalization of *ailanthus* in the United States.

Although total dry weight of American seedlings was less than that of Chinese seedlings, height and diameter and leaflet size indicated that inbreeding depression does not exist within American *ailanthus*. American seedlings were as genetically variable as Chinese seedlings. Consequently, two explanations are possible. First, no significant reduction of gene pool occurred when the species was introduced into the United States and second, mutation and recombination may have been factors involved in increasing genetic variance within the American populations if the initial gene pool was small. American seed sources were less diverse than Chinese seed sources while variance of individual trees within seed sources was greater. Thus, American *ailanthus* has undergone shifts in the distribution

of genetic variance among seed sources and individual trees since its introduction two centuries ago.

62. Feret, P.P. and G.R. Stairs. 1971.

Enzyme electrophoresis — application of molecular biology to forest genetics research. *In: Proceedings of the 18th Northeast Forest Tree Improvement Conference, Yale University, New Haven, Connecticut, August 2-4, 1970.* pp. 72-82.

Genetic variation in forest trees has traditionally been investigated using techniques of quantitative genetics. Allelic variation has not been intensively studied because of a general absence of useful gene markers. The technique of enzyme electrophoresis allows analysis of the enzyme products of an allele; hence, a promising method is available for the study of allelic variation in forest trees. The broad application of the technique for genetic analysis may be restricted by a number of factors including: allelic interactions, genic regulators, environmental alteration of enzyme expression and extraction artifacts.

An analysis of peroxidase isoenzymes in *U. pumila* seed sources indicates that peroxidase expression is controlled by a polymorphic locus with nine alleles. Genetic experiments will be required to validate this interpretation.

63. Feret, P.P. and G.R. Stairs. 1971.

Peroxidase inheritance in Siberian elm. *Forest Sci.* 17: 472-475.

Peroxidase isoenzyme analyses of S_1 and F_1 families of Siberian elm (*Ulmus pumila* L.) indicate that peroxidase patterns are controlled by a one-locus, three-allele genetic system.

64. Feret, P.P. and M.S. Witter. 1977.

GOT macrogametophyte isoenzymes of Virginia pine. *In: Proceedings of the 14th Southern Forest Tree Improvement Conference,*

Gainesville, Florida. June 14-16, 1977. pp. 255-260.

Analysis of populations of macrogametophytes from *Pinus virginiana* L. demonstrated genetic control at two loci for glutamate oxalo-acetate transaminase (GOT) isoenzymes. A comparison among four populations illustrated that natural stands and seed orchard progeny are not significantly different at the GOT locus A either in allelic composition or percentage of individuals heterozygous at the A locus.

65. Fins, L. and Libby, W.J. 1982.

Population variation in sequoiadendron: seed and seedling studies, vegetative propagation, and isozyme variation. *Silvae Genet.* 31: 102-110.

Seed samples were collected from 35 natural populations of giant sequoia and examined for seed weight, germination percent, cotyledon number, rootability of cuttings, and isozyme variation. Samples were significantly variable in percent seed germination, cotyledon number, isozyme allele frequencies, and observed heterozygosity. Seed germination varied among populations, but did not reveal any clear geographic patterns. Cotyledon numbers (of 871 seedlings) varied among populations and geographic areas. Cuttings (from 608 seedlings) rooted at 94 percent. Isozyme variation was found in every population sample at one or more loci. Little if any recent gene flow is likely to have occurred between the northern and southern populations. Relatively low heterozygosity among embryo samples suggests that inbreeding and/or population substructuring is likely in giant sequoia populations. Relatively higher levels of heterozygosity are found in the southern parts of the range, suggesting different local selective regimes. Early data suggest that the most northern native population (Placer Grove), may be substantially different from the other populations.

66. Florence, L.Z. and R.R. Hicks. 1980.

Further evidence for introgression of *Pinus taeda* with *P. echinata*: electrophoretic varia-

bility and variation in resistance to *Cronartium fusiforme*. *Silvae Genet.* 29: 41-43.

Twenty-nine single tree selections of loblolly (*Pinus taeda*), shortleaf (*P. echinata*), and suspected hybrid pines were made from natural populations in the species western-most distributions (East Texas). Electrophoretic assays of general proteins yielded 13 unique, electrophoretic phenotypes which were compared to the response exhibited by the open-pollinated progeny families to inoculation by *Cronartium fusiforme* (fusiforme rust). Results suggest that introgression of the two pine species has occurred and that maternal morphology and electrophoretic phenotype represent reasonable methods for predicting progeny response to fusiforme rust among sympatric loblolly-shortleaf pine populations in East Texas.

67. Florence, L.Z. and G. Rink. 1979.

Geographic patterns of allozymic variation in loblolly pine. In: Proceedings of the 15th Southern Forest Tree Improvement Conference, Mississippi State University, June 19-21, 1977. pp. 33-41.

Haploid maternal gametophytes from more than 200 loblolly pine (*Pinus taeda* L.) trees sampled from eight geographic provenances were assayed for six enzyme systems. Ten allozyme loci were identified having an average of 3.6 alleles per locus.

Results indicated considerable genetic differentiation has evolved within the loblolly pine species. Structuring of the species genetic variation may be a result of restriction to gene flow and varying selection regimes over the natural range of loblolly pine.

68. Fowler, D.P. and R.W. Morris. 1977.

Genetic diversity in red pine: evidence for low genic heterozygosity. *Can. J. For. Res.* 7: 343-347.

Starch gel electrophoresis was used to survey for genetically determined enzyme mobility dif-

ferences among 297 megagametophytes of red pine (*Pinus resinosa* Ait.) from five widely separated geographical sources. Consistent and reproducible enzyme banding patterns were observed with five of the seven isozyme systems assayed. No variation in band mobility was observed in any of these systems. This result stands in contrast with those reported from surveys of allozyme variation in other coniferous species but is consistent with the low degree of genetic variation observed in red pine for higher levels of genetic organization. It is concluded that red pine is genetically depauperate.

Possible explanations for restricted genetic diversity are discussed. The most plausible explanation suggests that red pine was at sometime, possibly during the Pliocene, reduced to a small refugial population and has yet to reestablish equilibrium heterozygosity.

69. Friedman, S.T. and W.T. Adams. 1981.

Genetic efficiency in loblolly pine seed orchards. *In: Proc. 16th South. Forest Tree Improv. Conf.*, Blackburg, VA. pp. 213-224.

Using seven allozyme loci as genetic markers, gene frequencies in the bulked seed crop of two seed orchards were compared to those expected on the basis of maximum genetic efficiency. Significant deviations from expected values were found for allelic frequencies in embryos as well as in both the ovule and pollen pools which produced the embryos. While contamination from pollen sources in surrounding stands (estimated at 28%) may be important in causing deviations from expectation in the pollen pool, self-fertilization does not appear to be a problem since the proportion of selfed progeny was estimated to be very low (< 1.5%) in these orchards. When seeds were separated by size there was as substantial loss of genetic variability in the ovule pool within a size class. Several other factors which might be responsible for decreased genetic efficiency are considered. Implications of these findings for seed orchard management are discussed.

70. Gan, Y.Y.; F.W. Robertson; P.S. Ashton; E. Soepadmo and D.W. Lee. 1977.

Genetic variation in wild populations of rain-forest trees. *Nature*. 269: 323-325. [Forestry Abstracts 39, 1191].

Electrophoretic analysis was carried out on the leaves of 2 species — *Shorea leprosula* (an emergent species) and *Xerospermum intermedium* (an understory species) — collected mainly from 3 sites in Pasoh Forest Reserve, S. Peninsular Malaysia. Intra-specific genetic variation of isozymes was demonstrated and an analysis of variation in leaf morphology in *X. intermedium* between trees in relation to their distance apart showed significant heterogeneity. It is suggested that both species have an outbreeding system; this may be combined with short-range heterogeneity in gene frequency which may be related to short-range pollen and fruit dispersal, leading to physical isolation between populations had hence, speciation.

71. Grant, M.C. and J.B. Mitton. 1977.

Genetic differentiation among growth form of Engelmann spruce and subalpine fir at tree line. *Arctic and Alpine Research*. 9: 259-263.

Starch gel electrophoresis was used to assay for genetic variation in Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) near tree line in the Rocky Mountains of Colorado. Substantial differentiation of peroxidase enzymes was detected along an elevational gradient in samples marked by different growth forms. The genetic differentiation observed runs counter to a commonly held view that extensive gene flow among these wind pollinated long-lived plants would maintain genetic homogeneity. The differentiation among peroxidase enzymes may be related to their fungicidal properties and the occurrence of snow molds in spruce and fir near the tree line.

72. Grant, M.C.; Mitton, J.B. and Linhart, Y.B. 1982.

Ecological and evolutionary studies of forest trees in Colorado. *In: Ecological Studies in*

the Colorado Alpine: A Festschrift for John W. Marr. Edited by J.C. Halfpenny, University of Colorado. p. 96-100.

Forest tree components of the Colorado Front Range ecosystems have recently been studied from several ecological and genetical perspectives. Among the chief results of this work are (1) microgeographic differentiation, even among these long-lived, wind-pollinated perennials, is common; (2) levels of genetic variability are high compared to herbaceous vegetation; (3) temporal genetic differentiation; is weak compared to spatial differentiation, (4) overall levels of protein heterozygosity are often correlated with radial growth rates and/or radial growth rate variability; (5) age and size structures of ponderosa, limber, and lodgepole pine and Engelmann spruce show significantly different distributions which, in turn, appear to correlate with the successional status of each species; (6) aspen clones show strong patterns of differentiation in adult sex ratios along an elevational gradient; (7) Engelmann spruce and Colorado blue spruce show no signs of hybridization as assessed by protein phenotype patterns; and (8) significant differences in morphology and protein genotypes were detected among mountain pine beetles which emerged from their different host tree species.

73. Gullberg, U.; Yazdani, R. and Rudin, D. 1982.

Genetic differentiation between adjacent populations of *Pinus sylvestris*. *Silva Fenn.*, 16: 205-214.

The possibilities are considered, in terms of Wright's theory of shifting balance, for the rapid evolution of Scots pine populations under natural conditions in Sweden.

The study is concerned with three adjacent stands in the southernmost part of the Swedish mountain range and with two such stands outside Stockholm.

In the mountain range a stand exposed to wind has later flowering periods than surrounding stands. Outside Stockholm a stand in a bog also has later flowering than the dominating stand

types. It is shown that these differences cause a partial genetic isolation despite high rates of pollen entering these sites from outside.

The partial isolation and indications of diversifying selection in the adjacent stands have led us to maintain that situations could be identified for Scots pine where the theory of shifting balance is valid.

Isozyme techniques were used to study the genetic polymorphism within and between three stands from the mountain range. The result of this study points out that genetic differentiation has occurred between these adjacent stands.

74. Guries, R.P.; S.T. Friedman and F.T. Ledig. 1978.

A megagametophyte analysis of genetic linkage in pitch pine (*Pinus rigida* Mill.). *Heredity*, 40: 309-314.

The haploid state of conifer megagametophyte tissue provides a means of testing for genetic linkage among allozyme loci in conifers. Using pitch pine trees (*Pinus rigida* Mill.), which were previously determined to be heterozygous for certain allozymes, we have been able to detect linkage among several pairs of loci. One such pair, GOT-1 and GPI-2, is tightly linked showing a recombination frequency of 4 percent. Four other pairs appear to be linked with recombination frequencies at the margin of detectability, 38-40 percent, but tree-to-tree variation makes positive determination difficult for less tightly linked loci. Such variation may be due to small sample sizes and presence of inversion polymorphisms. These results are the first report of tight linkage in a forest tree and represent a start in mapping the conifer genome.

75. Guries, R.P. and F.T. Ledig. 1977.

Analysis of population structure from allozyme frequencies. In: *Proceedings of the 14th Southern Forest Tree Improvement Conference*, Gainesville, Florida. June 14-16, 1977. pp. 246-253.

Selection strategies for tree improvement depend on population structure which will probably vary among species and even among regions within a species' range. Analysis of population structure using allozyme information can help in determining improvement strategy. Population structure can be described in terms of inbreeding, the grouping of genotypes within stands, and genetic distances among stands.

There was no evidence of inbreeding in stands of pitch pine based on analysis of 15 allozyme loci, nor was there evidence of familial groups within stands. However, there were differences among stands over distances of several kilometers. The implication for tree improvement strategy in pitch pine is to use comparison-tree methods of selection, and for provenance testing, to expend little effort on sampling different stands within regions.

76. Guries, R.P. and F.T. Ledig. 1978.

Inheritance of some polymorphic isoenzymes in pitch pine (*Pinus rigida* Mill.). *Heredity*, 40: 27-32.

The inheritance of eight polymorphic isoenzyme systems encoded by 14 loci is demonstrated in pitch pine using megagametophytes and embryos of open-pollinated seed collected in natural stands. Electrophoretic variants of isocitrate dehydrogenase and aconitase segregate in megagametophytes in a pattern suggestive of control by a single locus. The segregation of two zones of activity on gels stained for 6-phosphogluconate dehydrogenase, malate dehydrogenase, phosphoglucomutase, glucose phosphate isomerase and leucine aminopeptidase suggests that two loci control each of these enzymes. The finding that electrophoretic variants in each zone segregate randomly with respect to the other zone supports this hypothesis. Three zones of activity are observed on gels stained for glutamate-oxaloacetate transaminase with electrophoretic variants at GOT-2 coinciding precisely with variants at the cathodally migrating zone. Whether both zones are controlled by the same locus or whether they represent two tightly linked loci remains unknown. No evidence of genetic linkage was observed between pairs of loci that were heterozygous in the same mother-tree.

77. Guries, R.P. and F.T. Ledig. 1979.

Genetic structure of populations and differentiation in forest trees. In: *Proceedings of Symposium on Isozymes of North American Forest Trees and Insects*, July 27, 1979, Berkeley, Calif. Gen. Tech. Rep. PSW-48. Pacific Southwest Forest and Range Exp. Sta., Forest Serv., U.S. Dep. Agric., Berkeley, Calif., pp. 42-47.

Electrophoretic techniques permit population biologists to analyze genetic structure of natural populations by using large numbers of allozyme loci. Several methods of analysis have been applied to allozyme data, including chi-square contingency tests, F-statistics, and genetic distance. This paper compares such statistics for pitch pine (*Pinus rigida* Mill.) with those gathered for other plants and animals. On the basis of these comparisons, we conclude that pitch pine shows significant differentiation across its range, but appears to be less differentiated than many other organisms. Data for other forest trees indicate that they, in general, conform to the pitch pine model. An open breeding system and a long life cycle probably are responsible for the limited differentiation observed in forest trees. These conclusions pertain only to variation at allozyme loci, a class that may be predominantly neutral with respect to adaptation, although gene frequencies for some loci were correlated with climatic variables.

78. Guries, R.P. and F.T. Ledig. 1982.

Genetic diversity and population structure in pitch pine (*Pinus rigida* Mill.). *Evolution*, 36: 387-402.

Genic diversity and the organization of genetic variability in pitch pine were examined in 11 populations across the species range. Pitch pine is genetically variable; 76.2% of the loci studied were polymorphic and the average individual heterozygosity was 14.6%, but this is less than that exhibited by several other woody species. The contrast may reflect differences among species in their evolutionary history, or may be an artifact relating to the choice of enzyme systems analyzed.

Only a small percent of the observed genic diversity in pitch pine appears to be interpopulation, the remainder is due to differences between individuals within populations, in agreement with results for other tree species. Analysis of F-statistics indicates that populations of pitch pine simulate panmixis with an F_{15} of 0.009. Populations are only weakly differentiated, and genetic and geographic distance are only weakly correlated.

Central populations seem to be more variable than those near the species border. An isolated population at the northern extreme of the species range has a heterozygosity of 11.7%, compared to the species average of 13.8% and an average of 15.0% for central populations from New Jersey.

The dwarf forest populations of the New Jersey Pine Plains are essentially identical in genic constitution to tall forest of the New Jersey Pine Barrens, at least for the allozyme loci we sampled. Whatever factors are responsible for the dwarf stature of these populations, they have not resulted in detectable changes in allozyme frequencies among populations. In general, results of allozyme analysis are not in agreement with previously reported patterns for morphological and growth traits, emphasizing the need to sample several types of loci to adequately understand the genetic structure of populations.

79. Guzina, V. 1974/75.

Genetic polymorphism of the isoenzymes of peroxidase and esterase in *Populus deltoides*. Topola, 18/19: 170-176. [Forestry Abstract 37, 1439].

Reports the frequency of occurrence of 6 isoperoxidases and 2 isoesterases in the leaves of 34 half-sib families, from 11 States of the USA, grown at Novi Sad, Yugoslavia. The results are likely to be useful for identifying clones.

80. Guzina, V. 1974.

Isoenzymes of peroxidases in genetic studies of Poplar. Genetika, 6:63-68. [Forestry Abstracts 37, 109].

Zymograms of isoperoxidases, obtained by starch-gel electrophoresis, were prepared for 93 three-month-old seedlings of a half-sib family of *Populus tremula*. The 14 combinations of presence and intensity of seven isoperoxidase bands that were recognized appeared to represent 14 distinct genetic groups of plants. It is suggested that this approach may be useful for the identification of clones and in the study of the variability of hybrid families and natural populations.

81. Hamaker, J.M. and E.B. Snyder. 1973.

Electrophoresis patterns of needle enzymes in longleaf and sonderegger pines. U.S.D.A. For. Serv., Res. Note. SO-151. Southern Forest Experiment Station. 8 p.

Screening of 53 enzymes revealed 19 that were active and nine of these that were potentially useful for genetic studies of longleaf pine and its hybrids.

82. Hamrick, J.L.; J.B. Mitton and Y.B. Linhart. 1979.

Levels of genetic variation in trees: Influence of life history characteristics. In: Proceedings of Symposium on Isozymes of North American Forest Trees and Insects, July 27, 1979, Berkeley, Calif. Gen. Tech. Rep. PSW-48. Pacific Southwest Forest and Range Exp. Sta., Forest Serv., U.S. Dep. Agric., Berkeley, Calif., pp. 35-41.

In a previous study, levels of genetic variation, as measured by isozyme analyses, were compared for 113 taxa of vascular plants. Each species was classified for 12 life history and ecological traits and three measures of genetic variation were calculated. Plants with large ranges, high fecundities, an outcrossing mode of reproduction, wind pollination, a long generation time, and from habitats representing later stages of succession tended to have more isozyme variation than species with other combinations of characteristics. This paper discusses the results of the previous study and examines the available isozyme data for similar trends in forest trees. Special consideration was given to differences in

genetic variation among 20 conifer species that have many of their life history characteristics in common. Successional stage, habitat type, cone type and historical events were associated with differences in genetic variation among the conifer species. These results are discussed in terms of expectations from current population genetics theory.

83. Hare, R.C. and G.L. Switzer. 1969.

Introgression with shortleaf pine may explain rust resistance in western loblolly pine. U.S.D.A. For. Serv. Res. Note. SO-88. Southern Forest Experiment Station. 2 p.

Electrophoretic patterns of seed proteins and similarities in morphological characteristics suggest introgression between western sources of loblolly pine (*Pinus taeda* L.) and shortleaf pine (*P. echinata* Mill.). Fusiform rust resistance of western loblolly may arise from such introgression.

84. Harry, D.E. 1983.

Identification of a locus modifying the electrophoretic mobility of malate dehydrogenase isozymes in incense-cedar (*Calocedrus decurrens*), and its implications for population studies. *Biochem. Genet.* 21: 417-434.

Using megagametophyte (maternal haploid) and embryo (diploid) tissues of incense-cedar seeds, the expression of one of three malate dehydrogenase (MDH) loci was found to be influenced by a second, unlinked, modifier locus. Whereas alleles of the affected structural locus are codominant, the modifier alleles show dominance. The action of the modifier, limited to one of 28 structural loci examined, results in a shift of electrophoretic mobility observable in conventional starch gels. Both the structural and modifier MDH loci are polymorphic in all populations surveyed. Studies of genetic variation in natural populations made without rigorous genetic analysis may not detect such modification. By misinterpreting the genetic basis of enzyme phenotypes, such undetected modification can result in overestimates of genetic diversity in natural

populations and can cause an apparent excess of homozygotes relative to expectations. These effects on allele and genotype frequency estimates are dependent on the levels of polymorphism at both the structural and the modifier loci. Using procedures common to many surveys of electrophoretic variation, the frequency of a recessive modifier allele could be as high as 0.3 before being detected.

85. Hattemer, H.H. 1978.

The importance for forestry of genetic diversity in forest trees. *Forstarchiv*, 49: 249-256. [Forestry Abstract 40, 1766].

The tendency for the genetic diversity of tree plantations to be reduced is illustrated by contrasting the number of clones of poplar (*Populus*) in W. German populations over the period 1955-75 with the considerably greater number of potato varieties under cultivation during this period. The potential use of polymorphic gene loci (e.g., those for isoenzymes) as markers for genetic selection is discussed, and illustrated by published data for Norway spruce (*Picea abies*) provenances.

86. Hattemer, H.H. 1979.

Problems in population genetic conclusions from biochemical marker studies. In: *Proceedings of the Conference on Biochemical Genetics Forest Trees*, Umea, Sweden, 1978. pp. 73-85.

The identification of gene loci in forest trees has gained considerable importance. Known gene markers almost exclusively are biochemical markers. In conifers the relative amount of some monoterpenes and sesquiterpenes in diploid tissue has been found to be controlled by single gene loci. Also in conifers, the genetic analysis of isozymes active in the seed and/or in the needles has proved to possess an enormous potential. The haploid status of the endosperm and the correspondence between endosperm and embryo contained in one and the same seed are typical of conifers and permit a variety of population genetic inferences. The nature of such inferences is briefly described.

87. Hiebert, R.D. and J.L. Hamrick. 1983.

Patterns and levels of genetic variation in Great Basin bristlecone pine, *Pinus longaeva*. *Evolution*, 37: 302-310.

Patterns and levels of genetic variation were studied in five bristlecone pine (*Pinus longaeva*) populations in the eastern Great Basin. Fourteen enzyme loci were examined using horizontal starch gel electrophoresis. Low but significant levels of genetic variation were observed among populations. No patterns of decreasing genetic identity were observed with an increase in geographic separation.

In contrast, levels of genetic variation within populations were high. All populations were polymorphic for the same 11 loci. Levels of heterozygosity were effectively the same in all populations with a mean heterozygosity of 0.327. Genetic identity between altitudinal zones within populations was effectively the same as that between populations.

Analyses were also done to determine if there were deviations in expected genotype frequencies. All but two of the loci deviated significantly from expectations in at least one population. Acp-4 had consistent excesses of heterozygotes while Gdh-3, Lap-8 and Got-5 have consistent deficiencies. All other polymorphic loci deviated from the expected in both directions.

The low interpopulation variation and the high intrapopulation variation, along with paleoecological data, argue for the continuity of bristlecone pine throughout much of the Great Basin during the last full glacial. The maintenance of high levels of variation from that time until the present are a result of several factors which would appear to include: (1) high outcrossing rates; (2) maintenance of large population sizes; (3) production of large numbers of seeds; (4) microhabitat adaptation in a spatially and temporally heterogeneous environment.

88. Hill, R.J.; G.T. Prance; S.A. Mori; W.C. Steward; D. Shimabukuru and J. Bernardi. 1978.

Electrophoretic study of the dynamics of genetic variation of three riverine species

along the Solimoes River, South America. *Acta Amazonica*, 8: 183-199 [Forestry Abstracts 41, 4461].

Aeschynomene sensitiva var. *sensitiva*, *A. sensitiva* var. *amazonica* and *Mimosa pigra* were studied along a 2174-km reach of the river, in Brazil and Peru. Genetic variation was assessed in terms of the degree of enzymatic polymorphism and the average population heterozygosity. A close relationship between genetic variability and adaptive strategy was observed.

89. Hopper, S.D. and G.F. Moran. 1981.

Bird pollination and the mating system of *Eucalyptus stoatei*. *Aust. J. Bot.* 29: 625-638.

Eucalyptus stoatei C. A. Gardn., a small tree endemic in south-western Australia, is unusual in the genus in being predominantly if not exclusively pollinated by birds. Many individual honeyeaters but no insects were recorded feeding on its nectar during the 1980 flowering season. The large pendulous flowers deny entry to most large insect visitors because the stamens form an impenetrable dome over the floral cup. Access to nectar is only possible through a narrow opening lined with anthers in the center of the staminal dome.

Honeyeaters visited few flowers per tree (average 1-5) and about 18% of the movements of individual birds from one flower to another were between flowers on different trees. Most intertree movements were between trees farther apart than nearest-neighbors. Hence a high rate of outcross pollinations is inferred. Analysis of the mating system of *E. stoatei* using allozyme seed markers in one population indicated an average level of outcrossing of 82%, among the highest so far recorded for eucalyptus. The study therefore supports the hypothesis that bird pollinators may effect high levels of outcrossing, although other factors such as protandry and incompatibility probably are also involved.

Adaptations favoring exclusive bird pollination in *E. stoatei* may have evolved to promote outcrossing and heterozygosity in the face of inbreeding depression imposed by a dissected population structure, and in response to the rela-

tively low number of seeds per plant produced by the species.

90. Houston, D.B. 1983.

Stand and seed source variation in peroxidase isozymes of *Quercus rubra* L. *Silvae Genet.* 32: 59-63.

Variability in *Quercus rubra* foliar peroxidase isozymes was studied in 11 seed sources and 3 local stand collections. Analysis of isozyme frequency distributions by Berry's measure of distinctiveness, D^2 , revealed significant differences for all but one (Michigan vs Pennsylvania) of the 55 pair-wise seed source comparisons. All sources could be tentatively differentiated from one another based on the presence or absence of one or more isozyme bands, or by large frequency differences between one or more isozymes.

Berry's D^2 also indicated the existence of significant differences in isozyme frequency distributions between trees (i.e., between four half-sib families) sampled in each of 3 Ohio red oak stands, as well as between stands.

91. Houston, D.B. and S.K. Hood. 1982.

Genetic variation in peroxidase isozymes of *Liriodendron tulipifera* L. *J. Hered.* 73: 183-186.

Peroxidase inheritance patterns for 10 full-sib *Liriodendron tulipifera* L. families were examined by acrylamide gel electrophoresis. Six of 13 isozymes detected were sufficiently stable and reproducible to be analyzed by chi-square methods. Isozyme segregation frequencies approximated Mendelian expectations in 47 of the 50 chi-square analyses performed. Each isozyme was found to be controlled by a single locus with two alleles, one dominant and one recessive (silent).

92. Jacobs, B.F.; C.R. Werth and S.I. Guttman. 1984.

Genetic relationships in *Abies* (fir) of eastern United States: an electrophoretic study. *Can. J. Bot.* 62: 609-616.

Abies (fir) is widely considered to consist of two species in the eastern United States. *A. balsamea* (L.) Mill. and *A. fraseri* (Pursh.) Poir., distinguished by relative cone bract to subtending cone scale lengths and number of leaf hypodermal cells. Intermediate individuals have been recognized as *A. balsamea* var. *phanerolepis* Fern. An understanding of the relationship between the two putative species hinges on the interpretation of the intermediate. Two alternative hypotheses view the intermediate as either an interspecific hybrid or as an intraspecific variant. Twenty gene loci were electrophoretically analyzed in samples from 12 populations representing all three taxa. All populations shared similar complements of alleles at comparable frequencies for 13 polymorphic loci. Genetic distances among populations ranged from 0.10 to 0.0. Cluster analysis joined populations of *A. fraseri* with those representing *A. balsamea* var. *phanerolepis* at a distance of 0.03 and linked populations of *A. balsamea* with the other two taxa at 0.06. A hierarchical analysis of the three taxa, delimited by cluster analysis and bract exsertion, places *A. balsamea* var. *phanerolepis* intermediate between the two species. These data support a conspecific status for all eastern U.S. *Abies* with gene exchange between populations relatively unrestricted at last contact (ca. 10 000 years BP). Further, *A. balsamea* var. *phanerolepis*, while electrophoretically intermediate, is likely not of hybrid origin. Our results are consistent with those of recent studies which conclude that *A. fraseri* and *A. balsamea* are very closely related taxa of Pleistocene origin.

93. Joly, R.J. and W.T. Adams. 1983.

Allozyme analysis of pitch X loblolly pine hybrids produced by supplemental mass-pollination. *For. Sci.* 29: 423-432.

Allozyme genetic markers were used to evaluate the effectiveness of supplemental mass-pollination for the production of pitch $\times$ loblolly pine

hybrids. On the basis of the frequency of six mass-pollinated clones of pitch pine (*Pinus rigida* Mill.), the average proportion of hybrid seed set was estimated as 0.11; however, that estimate varied considerably among clones (0.01-0.32). Proportions of hybrids in 1-year-old nursery-grown progeny, as estimated by morphological features, also varied widely among clones, but averaged 0.20. The discrepancy between the allozyme and nursery estimates and the low frequency of hybrids are discussed, emphasizing the potential usefulness of allozyme markers in future studies of supplemental mass-pollination.

94. Jonsson, I. 1979.

Isolation and partial characterization of isoenzymes of acid phosphatases from needles of *Pinus silvestris* L. In: Proceedings of the Conference on Biochemical Genetics of Forest Trees, Umea, Sweden, 1978. pp. 5-15.

Acid phosphatases were extracted from pine needles after homogenization, followed by a combined acetone and ammonium-sulphate precipitation procedure. The precipitate after solubilization was subjected to gelfiltration and preparative isoelectric focusing. After removal of the ampholines about 5% of the initial activity was recovered. The preparation was found to consist of two main isoenzyme forms, when subjected to Counter Current Distribution, electrophoresis and isoelectric focusing. Molecular and kinetic characteristics were very similar for both isoenzyme forms. The isoelectric points, however, differed 6 pH units, being 3,5 and 9,5 respectively.

Association of an endogenous low molecular weight factor with the basic main isoenzyme fraction yields the acidic main isoenzyme fraction. Dissociation of this complex is also demonstrated.

95. Juo, P-s and G. Stotzky. 1970.

Electrophoretic patterns of globulins and albumins in conifer seeds. Can. J. Bot. 48: 1911-1912.

The globulins and albumins in seeds of nine species of conifers were distinguished by disc electrophoresis. On the basis of their higher stain intensity, the slower moving proteins, located in the upper half of the separation gels, appeared to be dominant. Only one globulin and one albumin band were homologous in all species examined, although several globulin and albumin bands appeared to be common to many species. The degree of similarity in protein patterns between species classified within the same genus and the degree of difference between genera were not as great as those reported for seeds of some angiosperms.

96. Juo, P-s and G. Stotzky. 1973.

Electrophoretic analysis of isozymes from seeds of *Pinus*, *Abies*, and *Pseudotsuga*. Can. J. Bot. 51: 2201-2205.

Isozyme patterns of L-leucine-amino-peptidase (LAP), esterase, and peroxidase in extracts from conifer seeds (eight species of *Pinus*, *Abies concolor* Hoopes, and *Pseudotsuga taxifolia* Britt.) indicated that certain specific isozyme patterns might be useful in systematic studies of conifers. The isozyme patterns of LAP from these seeds showed an apparent phylogenetic relationship. Eight different LAP bands were detected, and all eight species of *Pinus* displayed the same three LAP bands. The two slowest moving LAP bands were found to be common to all 10 species, whereas the mobility of the other LAP bands varied among species. Isozyme patterns of esterase from seeds of *Pinus*, *Abies*, and *Pseudotsuga* varied from species to species, and no species displayed the same number of bands. The number of esterase bands detected varied from 5 to 16, and no phylogenetic relationships were apparent. The banding patterns of peroxidase from these conifer seeds, which ranged from three to nine bands, also appeared to be species specific, and no common trends or homologies were apparent.

97. Kelly, W.A. and R.P. Adams. 1977

Seasonal variation of isozymes in *Juniperus scopulorum*: systematic significance. Am. J. Bot. 64: 1092-1096.

The relationship of seasonal variation in isozymes to systematic studies at the infraspecific level is evaluated. Isozyme variation in peroxidases, and α -terpineol dehydrogenases was evaluated monthly for one year in *J. scopulorum* Sarg. Zymograms of α -terpineol dehydrogenases showed one nonvariable band. Isoperoxidases varied quantitatively but not qualitatively, this variation being correlated with seasonal growth and dormancy. Isoesterases showed qualitative variation, with two types of esterases being produced. One type showed seasonal variation and a second was nonvariable. The presence of two types of isoesterases may reflect differing physiological roles for each. The esterase results showed that care should be taken during investigations of isozymes at the population level to assure phenological similarity.

98. Kelly, W.A. and R.P. Adams. 1978.

Analysis of isozyme variation in natural populations of *Juniperus ashei*. *Rhodora*, 80: 107-134. [Genetics Abstract G10, 12875].

The study of isozymes can lead to an understanding of a number of biological problems. These problems include the evolution of populations, the transformation of genes and the regulation of gene expression. A study of the genetic isozyme variation in populations of *Juniperus ashei* Buch. is reported. The analysis give discordant results, each enzyme system studied exhibits different trends. Three hypotheses are advanced to explain the unusual disjunct clusterings of populations of *J. ashei*. The inconsistencies in the distribution of *J. ashei*, outlined by the above research, raises questions about the concept of breeding population size, gene flow and natural selection. The completion of preliminary isozyme studies is necessary before these questions can be answered. The use of isozyme banding as a taxonomic character, in *J. ashei* is not feasible and does not agree with the morphological and terpenoid information previously collected.

99. Kim, C.S. and S.B. Chung. 1974.

Variation in the patterns of isoperoxidase in the genus *Populus*. I. Variation in the patterns

of isoperoxidase in *P. alba*, *P. glandulosa* and *P. euramericana*. Research Report of the Institute of Forest Genetics, Korea, 11: 53-59. [Forestry Abstracts 37, 835].

Zymograms were prepared from the leaves of 23 trees of *P. alba* (naturalized in Korea), 8 trees of *P. glandulosa* (native to Kyounggi province), and one clone each of *P. deltoides*, *P. nigra*, *P. 'I-154'*, *P. 'I-214'*, *P. 'I-476'*, and the Belgian Poplar *P. 'V-211'*. All the species and hybrids could be distinguished by their isoperoxidase banding patterns, and in addition four types (described as A, B, C and D) were identified in *P. alba* and another four types (also A-D) in *P. glandulosa*.

100. Kim, C.S. and S.S. Kim. 1977.

Variation in the pattern of isoperoxidase in genus *Populus*. II. Patterns of isoperoxidase in the leaves of 15 clones of X *Populus alba-glandulosa* [*P. alba* × *P. glandulosa*]. J. of the Korean Forestry Society, 36: 1-4. [Forestry Abstracts 40, 2129].

The clones could be distinguished by their banding patterns on zymograms prepared by starch gel electrophoresis.

101. Kim, C.S.; S.K. Lee and Y.G. Park. 1973.

Variation in the patterns of isoperoxidase in diploid and tetraploid *Robinia pseudoacacia*. Research Report of the Institute of Forest Genetics, Korea, 10: 23-24 [Forestry Abstract 33, 7412].

Presents zymograms for the leaves of 8 diploid and 12 [colchicine-induced] tetraploid individuals and for the seedling progeny of one individual of each type. Wide variations were found, especially among the tetraploids; band patterns were not correlated with the five phenotypes of the tetraploids. The variation within the progeny suggests that cleistogamy may not be complete in this species.

102. Kim, Z.-S. 1979.

Inheritance of leucine aminopeptidase and acid phosphatase isozymes in beech (*Fagus sylvatica* L.). *Silvae Genet.* 28: 68-71.

Starch gel electrophoresis was used to study the inheritance patterns of leucine aminopeptidase and acid phosphatase in beech. Progenies of 15 wind pollinated families and 2 controlled crosses were investigated.

Two polymorphic loci, LAP-A and ACP-A, were identified with 4 alleles each. Although a silent allele was found at the LAP-A locus, the phenotypes of the two loci segregated according to Mendelian inheritance.

Comparison of the observed genotypic class frequencies over both loci with the expected (assuming free recombination) indicated no linkage between the two loci.

103. King, J.N. and B.P. Dancik. 1983.

Inheritance and linkage of isozymes in white spruce (*Picea glauca*). *Can. J. Genet. Cytol.* 25: 430-436.

Variation patterns for 26 loci of white spruce (*Picea glauca* (Moench) Voss) from 16 enzyme systems were studied using haploid megagametophyte tissue. Inheritance models for 17 polymorphic loci were formulated and tested for deviations from Mendelian segregation. Linkage analyses were conducted for 12 of the polymorphic loci in pairwise comparisons. Significant joint segregation was found for three pairs of loci: Pgi-2 with Aat-2, Gdh with Idh, and one of three families tested with Pgi-2 and 6 pg-2,3.

104. Knowles, P. and M.C. Grant. 1981.

Genetic patterns associated with growth variability in ponderosa pine. *Am. J. Bot.* 68: 942-946.

Variability in annual growth increment in *Pinus ponderosa* was measured and heterozygosity level estimated in order to examine the relationship

between genetic heterozygosity and phenotypic homeostasis in natural populations of a long-lived perennial plant. Pollen was collected from 190 ponderosa pine trees in the upper montane region of the Front Range in Colorado and four isozyme systems were resolved electrophoretically from these samples. Each tree was cored, aged, and each annual ring width measured. Statistics estimating within-tree variation in growth increment were then related to heterozygosity levels. The results indicated a significant positive association between level of heterozygosity and growth variability. Two speculations are offered to account for this relationship.

105. Knowles, P. and J.B. Mitton. 1980.

Genetic heterozygosity and radial growth variability in *Pinus contorta*. *Silvae Genet.* 29: 114-118.

The relationship between heterozygosity level and stability in the phenotypic character, annual radial growth, was examined within a population of *Pinus contorta*. Needle tissue was sampled from 152 lodgepole pines in the subalpine region of the Front Range in Colorado. Heterozygosity levels were determined from four electrophoretically detectable enzymes from the needle tissue. Each tree was cored, ages were estimated, and ring widths were measured. The coefficient of variation, a measure of dispersion in growth increment, was calculated for each tree and related to the level of heterozygosity. The results indicate that trees with overall high levels of heterozygosity have significantly less growth variability than trees with low levels of heterozygosity. When the loci were examined separately, however, there are no significant differences between the genotypic groups. This suggests a complex interaction among the four loci involved. Although the mechanism linking overall heterozygosity level to phenotypic variability remains unknown, these results add to the increasing evidence that a strong relationship exists between heterozygosity and phenotypic variability.

106. Kormutak, A. 1976.

Enzyme activity in unpollinated, incompatibly and compatibly pollinated nucelluses of

the lodgepole pine (*Pinus contorta* Dougl.). *Biologia, Czechoslovakia*, 31: 521-526. [Forestry Abstract 38, 2103].

In the context of biochemical differentiation with respect to hybridity barriers, studies were made to compare peroxidase, glucose-6-phosphate dehydrogenase and cytochromoxidase activities in three *P. contorta* nucellus groups: (1) unpollinated, (2) incompatibly pollinated (*P. contorta* × *P. mugo*), and (3) compatibly pollinated (*P. contorta* × *P. banksiana*). In (1), all enzyme activity was low. In (2), there was a slight increase in peroxidase and glucose-6-phosphate dehydrogenase activities, while cytochromoxidase activity was unchanged. In (3), the activity of all three enzymes reached a maximum.

107. Krzakowa, M. 1980.

Variability of Glutamate-Oxalate-Transaminase (GOT-2.6.1.1) isoenzymes in open-pollinated progeny of homozygous Scots pine (*Pinus sylvestris* L.) trees. *Acta Soc. Bot. Pol.* 49: 143-147.

The progeny of the two homozygous Scots pine (*Pinus sylvestris* L.) plus-trees was investigated as regards the GOT enzyme system to check the influence of 18 trees growing in the nearest neighborhood. The experiment proves cross-fertilization only in almost 40 percent.

108. Krzakowa, M. 1982.

Genetic differentiation of Scots pine populations. 1. Genotypes. *Silva Fenn.*, 10: 200-205.

The early provenance investigations described the Scots pine of the North part of Poland as a different in many morphological and physiological characters from that of the middle part of the country. Eight populations, tested previously in provenance experiments, were used in this study as the representative samples of Polish Scots pine forests. At least 30 trees were randomly chosen from each population. Each mother tree was examined in respect of five enzyme systems: leucine-aminopeptidase, glutamate oxalacetate-

transaminase, 6-phosphogluco-dehydrogenase, alcohol dehydrogenase and glutamate dehydrogenase. Interpopulational divergences, based on genotypic frequencies, were described by Hedrick's distances, Mahalanobis' distances and Canonical Analysis.

109. Krzakowa, M. and J. Szweykowski. 1979.

Variation of 6-PGD in the populations of Polish Scots pine (*Pinus sylvestris*). In: *Proceedings of the Conference on Biochemical Genetics of Forest Trees*, Umea, Sweden, 1978. pp. 86-98.

The polymorphism variability of 6-PGD enzyme system was studied in 8 natural populations of Polish *Pinus sylvestris* (265 trees were investigated individually). There are three different loci coding for 8 allozymes (two loci with 3 allozymes each, one locus with 2 allozymes). The genic and genotypic structures have been described and similarity indices calculated. The differentiation map based on genic and genotypic structure of similarities is discussed.

110. Krzakowa, M.; J. Szweykowski and A. Korezyk. 1977.

Population genetics of Scots pine (*Pinus sylvestris* L.) forests. Genetic structure of plus-trees in Bolewice near Poznan (West Poland). *Bull. Acad. Pol. Sci., Ser. Sci. Biol.* 25: 583-590. [Genetics Abstracts G10, 5753].

Genotypic structure of 12 various enzymatic loci of 22 plus-trees in a man-made Scots pine forest in Bolewice (ca 70 km N-W from Poznan) were determined by means of macrogametophyte assay method. On average, 35% of investigated loci per individual turned to be heterozygous.

111. Ladizinsky, D. and T. Hymowitz. 1979.

Seed protein electrophoresis in taxonomic and evolutionary studies. *Theor. Appl. Genet.* 54: 145-151.

Seed protein electrophoresis is increasingly being utilized as an additional approach for species identification and as a useful tool for tracing back the evolution of various groups of plants. This paper summarizes the main features of the seed protein profile — stability, uniformity and additive nature. In addition, the significance of this approach for resolving specific taxonomic and evolutionary problems is pointed out.

112. Langridge, P.; G.F. Moran and A.H.D. Brown. 1981.

Biochemical genetics of some seed proteins of *Pinus radiata*. *Biochem. Genet.* 19: 585-597.

In a high-salt soluble fraction of the total protein from single seeds of *Pinus radiata*, up to 45 polypeptides were resolved on SDS-polyacrylamide gels. At least one-fifth of these polypeptides showed variation between seeds. In the 27 000-29 000 dalton region, two polypeptides were inherited as codominant alleles at a single locus and were shown to assort independently of another seed protein locus and three allozyme loci. A survey of 120 individuals from the five known native populations of *P. radiata* in California detected only the 27K and 29K alleles at the locus. In all populations, the 29K allele predominated, and the two island populations were monomorphic for the 29K allele. The 27 and 29K dalton polypeptides were shown to have very similar amino acid sequences, and the allelic difference at this locus is most probably in the gene sequence of the polypeptide.

113. Larionova, A. YA. 1979.

Variation of electrophoretic spectra of larch needle enzymes. *Biologicheskikh Nauk*, 2: 97-100. [Forestry Abstract 41, 4441].

The average number of electrophoretic zones with peroxidase and esterase activity was significantly different in consecutive years (in 1975-77) in studies on fully grown short shoots of *Larix gmelinii* from Krasnoyarsk territory, USSR.

114. Larionova, A. YA. and L.I. Milyutin. 1981

Investigation of the intraspecific differentiation of Siberian larch with the aid of isozyme spectra. *Lesovedenie*, 2: 3-11. [Forestry Abstracts 42, 5410].

A study was made on material from five provenances of *Larix sibirica* growing in trial plots near Krasnoyarsk. Details are given in tables and graphs of the intraspecific variability of isozymes of peroxidase and esterase in the needles. Considerable differences were found between some of the provenances, while geographically close populations had similar patterns of distribution of enzyme zone frequencies.

115. Ledig, F.T. and M.T. Conkle. 1983

Gene diversity and genetic structure in a narrow endemic, Torrey pine (*Pinus torreyana* Parry ex Carr.). *Evolution*, 37: 79-85.

Early investigations suggested that genetic variability might be associated with longevity, and that tree species were the most heterozygous of organisms. However, these ideas may be modified as species representing a wider range of population structure are investigated. Torrey pine, a California endemic, occurs in only two populations, one on the coast at San Diego and one 280 km northwest on Santa Rosa Island. Populations number only ca. 7 000 and 2 000 individuals, respectively. Each population is composed of identical homozygous genotypes at 59 isozyme loci. The island population is fixed for alternate alleles to those in the coastal population at two of the 59 loci. These results agree with other lines of evidence that suggest Torrey pine is characterized by minor differences between relatively uniform populations.

Drift is the most likely explanation for lack of variability in Torrey pine. It probably experienced one or more reductions in population size, most recently during the Xerothemic period 8 500 to 3 000 B.P. The founder effect may have operated during colonization of Santa Rosa Island ca. 18 000 B.P. Apparently, the Northern Channel Islands to which Santa Rosa belongs were not connected to the adjacent mainland in recent geo-

logic history, and colonization would require transport across at least a 6- to 7-km channel.

Red pine and western red cedar, conifers with greater ranges than Torrey pine, are only slightly more variable. Thus, despite the presence of mechanisms to maintain high levels of outbreeding, it appears the conifer breeding system can survive inbreeding with its consequence of genetic depauperization.

116. Ledig, F.T.; R.P. Guries and B.A. Bonfeld. 1983.

The relation of growth to heterozygosity in pitch pine. *Evolution*. 37: 1227-1238.

Within some populations, mean annual growth rate was greater for trees heterozygous at a high proportion of their isozyme loci than for relatively homozygous trees. The strength of the heterozygosity-growth relationship varied from strongly positive in the oldest stands to negative in the youngest. The regression coefficient relating growth to heterozygosity was strongly correlated with age ($r = .85$). Because of the record left by the annual rings, tree growth at various past ages can be related to heterozygosity, and within most stands the relationship between growth rate and heterozygosity increased as trees aged. Apparently, the superiority of heterozygotes is not expressed in young stands. The delayed expression of heterozygote superiority may reflect the greater homeostasis of heterozygotes in the face of year-to-year climatic variation, or the accentuation of differences by competition between heterozygotes and homozygotes as the canopy closes.

In fact, the heterozygosity-growth relationship was strongest in the least predictable environments ($r = .69$), as measured by the standard deviation of mean monthly maximum temperatures over years of record. But competition may also be involved because heterozygote superiority was most strongly expressed in the densest stands ($r = .28$). The failure of previous investigations to find correlations may result from scoring too few loci to adequately characterize heterozygosity, or to sampling populations in which competition was not strongly expressed. The relationship of growth to heterozygosity appears explicable with reference to age, stand structure, and climatic variability.

There was no heterosis associated with any single locus, suggesting that depression of growth in homozygotes is a result of linked deleterious recessives. Deleterious recessives are in high frequency in most conifers. Homozygosity in pitch pine probably identifies individuals carrying chromosome segments identical by descent from recent ancestors, and apparent heterozygote superiority may actually measure inbreeding depression in homozygotes.

Homeostasis in forest trees has been measured by the inverse of variability in ring width or annual increment. For pitch pine there was no consistent relationship of variability to heterozygosity, perhaps because of random error and because variability in annual increment is not a cumulative characteristic like mean annual increment.

117. Lewis, R.A. and F.C. Cech. 1969.

Electrophoresis separation of general protein and isoenzymes of black cherry seed (*Prunus serotina* Ehrh.). In: Proceedings of the 10th Southern Conference on Forest Tree Improvement, Houston, Texas, June 17-19. pp. 140-148.

It appears that there is a high degree of uniformity in acid phosphatase, leucine amino peptidase and peroxidase systems in trees of the West Virginia seed source.

Although esterase and general protein gels displayed numerous qualitative pattern variations, there appears to be a basic set of bands for each of these systems. These basic patterns were found to be stable in most trees tested.

On the basis of pattern resolution, peroxidase, esterase, and general protein were found to be the most promising systems for further study.

118. Linhart, Y.B.; M.L. Davis and J.B. Mitton. 1981.

Genetic control of allozymes of shikimate dehydrogenase in ponderosa pine. *Biochem. Genet.* 19: 641-646. [A note].

Mendelian inheritance of variation for shikimate dehydrogenase variation is demonstrated for ponderosa pine. In both ponderosa pine and in Douglas-fir three alleles are seen segregating in populations near Boulder, Colorado, and allelic frequencies are heterogeneous among localities.

119. Linhart, Y.B.; J.B. Mitton; D.M. Bowman; K.B. Sturgeon and J.L. Hamrick. 1979.

Genetic aspects of fertility differentials in ponderosa pine. *Genetic. Res., Camb.* 33: 237-242.

There is a strong genetic component to fertility differentials among individuals of ponderosa pine. Prolific cone-producers as a group were markedly different from low cone-producers at the three protein loci which were monitored. The two groups did not differ significantly in age, but trees with high cone production had slower growth rates and smaller diameters than trees with low cone production. To our knowledge, these results provide the first demonstration of fertility differentials associated with specific genes in a woody plant.

120. Linhart, Y.B.; Mitton, J.B.; Bowman, D.M.; Sturgeon, K.B. and Hamrick, J.L. 1979.

Some genetic consequences of differential reproduction in populations of forest trees. *In: Flowering and Seed Development in Forest Trees*, May 15-18, 1978, Mississippi State University. U.S. Forest Service, Southern Forest Experiment Station, Storkville, Miss., pp. 41-50.

Reproducing and non-reproducing individuals were compared for their genetic make-up in two populations of Ponderosa pine (*Pinus ponderosa*) and one population of Douglas-fir (*Pseudotsuga menziesii*) in Colorado, U.S.A. The genetic make up was determined for several protein loci in needle tissue of mature trees, using horizontal starch gel electrophoresis. There were some genetic differences in ponderosa pine and none in Douglas-fir. A detailed analysis of genetic dif-

ferences associated with differential reproduction was carried out in one population of ponderosa pine.

There are dramatic genetic differences between prolific cone producers and less productive individuals. The results have both practical and theoretical implications.

121. Linhart, Y.B.; J.B. Mitton; K.B. Sturgeon and M.L. Davis. 1979.

An analysis of genetic architecture in population of ponderosa pine. *In: Proceedings of Symposium on Isozymes of North American Forest Trees and Insects*, July 27, 1979, Berkeley, Calif. Gen. Tech. Rep. PSW-48. Pacific Southwest Forest and Range Exp. Sta., Forest Serv., U.S. Dep. Agric., Berkeley, Calif., pp. 53-59.

Patterns of genetic variation were studied in three populations of ponderosa pine in Colorado by using electrophoretically variable protein loci. Significant genetic differences were found between separate clusters of trees and between age classes within populations. In addition, data indicate that differential cone production and differential animal damage have genetic components. These results suggest that many diverse phenomena can affect allele frequencies within populations and may contribute to the high levels of genetic variability detected within populations of this species.

122. Linhart, Y.B.; J.B. Mitton; K.B. Sturgeon and M.L. Davis. 1981.

Genetic variation in space and time in a population of ponderosa pine. *Heredity*, 46: 407-426.

The genetic structure of a population of *Pinus ponderosa* was studied using seven electrophoretically-detectable protein loci. This population is composed of six groups of trees, which differ significantly from each other in their genetic constitutions. The results imply that the population is composed of groups of individuals assembled into genetically-related family units. Whenever

such family clusters have been documented in other studies, marked inbreeding has also been noted as a consequence. In this population, no inbreeding is detectable. The groups also differ from one another in several characteristics which have genetic consequences within the population as a whole. These characteristics include age structures, seed output, level of infestation by wooly aphids and extent of damage by deer browsing. The population was also divided into four age classes which do not differ markedly from one another in their genetic characteristics. This suggests that genetic differentiation in time is much less marked than differentiation in space.

123. Lundkvist, K. 1974.

Analysis of linkage in *Picea abies* by means of isozyme studies. In: Proceedings, Joint IUFRO Meeting, S.02.04.1-3, Stockholm, 1974. [Special Report]. pp. 468.

124. Lundkvist, K. 1974.

Inheritance of leucine aminopeptidase isozymes in *Picea abies* K. *Hereditas*, 76: 91-96.

Inheritance of leucine aminopeptidase isozymes in *Picea abies* K. was investigated by analyzing 6 mother-trees, progenies after selfing of all the mother-trees and progenies derived from 7 crosses. The isozyme variation in one specific region was due to a multi-allelic locus.

125. Lundkvist, K. 1975.

Inheritance of acid phosphatase isozymes in *Picea abies*. *Hereditas*, 79: 221-226.

The inheritance of acid phosphatase isozymes in *Picea abies* K. was studied by analyzing five parental trees and their offspring after self-pollination and various crosses. In the zymograms one multi-allelic locus with at least three alleles was detected. The structure of the isozymes seems to be dimeric.

126. Lundkvist, K. 1977.

Inheritance of esterases in needles and endosperms of Norway spruce (*Picea abies* K.). *Hereditas*, 87: 27-32.

Needles and seeds from 15 different clones of *Picea abies* were used in analyses of esterase isozymes. Four polymorphic esterase loci could be detected in preparations of endosperms and one locus in needle extracts, using starch-gel electrophoresis. The inheritance of isozymes in the latter locus was studied in 11 crosses between the clones. Although some obscurities in the inheritance of esterase isozymes are noted, the major part of the crosses indicates a Mendelian inheritance from parental clone to progeny.

127. Lundkvist, K. 1979.

Allozyme frequency distributions in four Swedish populations of Norway spruce (*Picea abies* K.). *Hereditas*, 90: 127-143.

Allozyme variation in eleven loci was studied in four Swedish populations of *Picea abies*. For six of the loci, GDH-B, GOT-A, GOT-C, LAP-A, MDH-A and MDH-B, the inheritance of allozyme bands was ascertained from their distributions to female gametes within single trees. Significant divergence in gene frequencies among populations was demonstrated, which in four of the loci appears to be correlated to differences in altitude of the habitats. Estimates of genetic variability was, however, uniformly distributed among populations. The possible influence of different selection intensities and adaptation to microhabitat was discussed. It was, furthermore, demonstrated in analyses of linkage that three of the allozyme loci studied are situated on one chromosome sufficiently close to reduce the frequency of recombination in female gametes. Finally, from preliminary studies of the mating system were obtained maximum estimates of self-fertilization in three of the populations.

128. Lundkvist, K. 1979.

Genetic differentiation within and among Swedish populations of Norway spruce (*Picea*

abies). In: Proceedings of the Conference on Biochemical Genetics of Forest Trees, Umea, Sweden, 1978. pp. 113-117.

The distribution of Norway spruce in Sweden covers about 12° in latitude and several hundreds of meters in altitude. Adaptation to habitat, which thus may differ significantly in length of vegetation period and climatic factors, will bring about a genetic differentiation among populations, the effects of which may be studied in provenance trials. One of the major topics in the allozyme studies of Norway spruce in Sweden has been to establish to what extent such differentiation is expressed in the isozyme patterns of natural populations.

129. Lundkvist, K. and D. Rudin. 1977.

Genetic variation in eleven populations of *Picea abies* as determined by isozyme analysis. *Hereditas*, 85: 67-74.

Eleven populations of *Picea abies* in Sweden were analyzed for isozyme variation in four polymorphic loci. Genetic differentiation between geographically isolated populations could be demonstrated, the major part of genic differences occurring in the esterase-A and acid phosphatase-A loci. When populations originating from foreign seeds are involved in comparisons with indigenous populations, higher values of genetic differentiation are found.

Genic variability within populations was estimated from "average heterozygosity", a measure which is based upon the observed gene frequencies. An increase in "average heterozygosity" was found in two populations of foreign origin, which to a certain extent may be due to intercrossing between genomes of different origin.

130. McMullan, E.E. and Colangeli, A. 1982.

Comparison of starch and polyacrylamide gel-electrophoresis and isoelectric focusing for isozyme analysis in two conifers. *Silva Fenn.*, 16: 226-231.

Experiences at the Pacific Forest Research Centre, Canada, with the use of starch and po-

lyacrylamide gel electrophoresis and isoelectric focusing for isozyme analysis are discussed. Results of terpene analysis, and leucine amino peptidase analysis by isoelectric focusing, indicate that the two techniques show similar population groups though there was no individual tree correlation. The possibility of computerized data recording for isozyme separations is discussed.

131. Mamaev, S.A. and L.A. Semkina. 1978.

Study of the intra-population variability of Scots pine by means of electrophoresis of seed proteins on polyacrylamide gel. In: Population structure and hardness of plants on the Urals. *Trudy Instituta Ekologii Rastenii: Zhivotnykh* (1978) No. 116, pp. 63-69. [Forestry Abstracts 49, 2538].

Studies were made on seeds from 16 trees, 40-50 yr old, at the southern end of the species' range in the Pre-Ural steppe (Chelyabinsk Province, USSR). Some 15 main and 9 subsidiary protein fractions were identified.

132. Matheson, A.C. 1980.

Unexpectedly high frequencies of outcrossed seedlings among offspring from mixtures of self and cross pollen in *Pinus radiata* D. Don. *Aust. For. Res.* 10: 21-27.

Mating is usually assumed to be random in *Pinus radiata* seed orchards, but these orchards normally contain many ramets of the same clones, leading to the possibility of extensive and undesirable self-fertilization. There is much evidence that inbreeding reduces both seed yield and seedling performance since inbred trees do not grow as well as outbred trees. Experiments were carried out with two marker genes. One was a recessive gene affecting the apical dominance and bud formation of seedlings. The other was a codominant gene affecting the net electrical charge on a seed enzyme. In crosses involving self and outcross pollen, fewer selfed seedlings occurred than expected. This could result from preferential outcrossing, or increased mortality among the inbred zygotes.

133. Matheson, A.C. and J.C. Bell. 1980.

Breeding systems in some central American pine species: A preliminary analysis. In: The IUFRO Symposium "Fast Growing Trees", August 25-30, 1980, Sao Paulo, Brazil. 9 p.

Only recently has it become possible to include the study of breeding systems in the catalogue of basic biology of forest trees. This has come about with the advent of gel electrophoresis of allozymes (enzymes produced by different alleles of the same gene locus). These techniques were used to study *Pinus caribaea* var. *bahamensis* (two stands), *P. caribaea* var. *hondurensis* (three stands), and *Pinus oocarpa* (five stands) in the Bahamas, Belize, Honduras and Nicaragua. Altogether eight gene loci were studied, each giving estimates of the outcrossing rate (t), Wright's fixation index (F), heterozygosity and population allele frequencies. In this preliminary analysis, only small numbers of seed per mother tree were examined and this had led to considerable variation in the estimates of t and F for each stand. However, the average value of the outcrossing rate (t) was found to be roughly between eighty and ninety percent indicating an inbreeding rate of between ten and twenty percent. The average value of Wright's fixation index was greater than zero indicating there were fewer heterozygotes than expected under random mating. This can arise if there is significant inbreeding or if there is heterogeneity in the pollen pool. The latter can occur where the sampled mother trees are far apart, or if there is neighborhood inbreeding. In a domestication program, neighborhood inbreeding effects resulting from a heterogeneous pollen pool disappear in one generation. Significant inbreeding not resulting from neighborhood effects is a more serious problem which could require modification of the domestication program. Since this is a preliminary analysis, it is not possible to be sure of the exact level of inbreeding, or of its cause. When more data is available, a fuller treatment will be carried out.

134. Matsuura, T. and K.-I. Sakai. 1972.

Geographical variation on an isozyme level in *Abies sachalinensis*. In: Proceedings of the

Joint Symposia for Forest Tree Breeding of Genetics Subject Group, IUFRO, and Section 5, Forest Trees, SABRAO. Tokyo, Japan, Government Forest Experiment Station of Japan. A-9 (V), 1-12.

Six populations of *Abies sachalinensis* growing wild in Hokkaido, Japan were investigated with regard to variation of individual trees in the electrophoretic zymograms of peroxidase. Compared among populations were average number of isozyme bands per tree and its intra-forest variability, number of lost or fixed bands, incidences of each band and the disagreement count among individual trees. Assuming that a population maintaining higher variability would be found in the neighborhood of the central area of distribution of the species, it has been concluded that the S population was a population probably close to the center of distribution, from which N population in one direction, and P, K and H in the other, had dispersed.

135. Mayberry, J.S. and P.P. Feret. 1977.

Peroxidases in developing acorns and seedlings of *Quercus alba* L. *Silvae Genet.* 26: 218-223.

Peroxidase isoenzymes have been shown to vary between organs and between trees in white oak. The isoenzymes differ both in total number of isoenzymes per organ and the relative mobilities of the isoenzymes. The amount of isoenzyme variation per organ is independent of isoenzyme patterns for other organs along a developmental sequence. Some organs (epicotyls and hypocotyls) have nearly identical isoenzyme patterns. Leaves and cotyledons showed the greatest amount of isoenzymes variation.

Of the tissues assayed, three hold promise for use in future studies. In maturing acorns, embryos yield reproducible isoenzymes and clear resolution. Seedling epicotyls represent a choice organ with high isoenzyme reproducibility and resolution. Hypocotyls are similar to epicotyls with the disadvantage of being available only during a limited period.

136. Mayberry, J.S.; P.P. Feret and R.E. Adams. 1976.

Peroxidase variation in *Quercus*. Virginia Journal of Science, 27: 58. [Plant Breeding Abstracts 47, 6796].

Between-tissue and between-tree differences for isoperoxidase enzymes were detected by polyacrylamide disc electrophoresis of acorns, and of root tips, hypocotyls, cotyledons, stem sections and leaves of seedlings grown from acorns, collected at weekly intervals from three *Q. alba* trees.

137. Mejnartowicz, L. 1976.

Genetic investigations on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) populations. Arboretum Kornickie, 21: 125-187.

On a provenance area at Kornik near Poznan the following characters were investigated in 100 Douglas-fir populations from the IUFRO 1966/67 series: height and increment rate, tree diameter, number of 1st order branches, dimensions of largest branch, phenology and frost hardness of trees as well as differentiation of some populations with respect to the frequency of occurrence of the LAP isoenzyme. Very significant interpopulational differences have been found for the majority of characters and economic usefulness of only a small number of populations. The following populations are regarded as best in respect of economy under conditions prevalent in Wielkopolska: 1047-Concrete and 1053-Darrington from the north-western part of Washington State.

138. Mejnartowicz, L. 1979.

Genetic variation in some isoenzyme loci in Scots pine (*Pinus sylvestris* L.) populations. Arboretum Kornickie, 24: 91-104.

Female gametophytes from seeds of 19 Scots pine (*Pinus sylvestris*) from Poland, FRG, USSR, Turkey and Hungary were analyzed for the content of leucine aminopeptidase (LAP) and acid

phosphatase (APH) isozymes. A considerable variability of APH enzyme was observed, with 15 alleles coding the APH-B locus. There were two LAP loci, LAP-A coded by 5 alleles and LAP-B coded by 6 alleles. A considerable genetic distance was observed between the Turkish and Hungarian populations. These populations from the southern part of the range differ substantially from other European ones, the Niepolomice population from southern Poland being intermediate. The variability of Scots pine proved to be much greater along the N-S axis than along the W-E axis. The populations from the Mazury region known for their great adaptability are characterized by considerable heterozygosity.

139. Mejnartowicz, L. 1980.

Polymorphism at the LAP and GOT loci in *Abies alba* Mill. populations. Bull. Acad. Pol. Sci. Ser. Sci. Biol. 27: 1063-1070. [Genetics Abstracts G13, 3909].

In the five Polish populations studied, leucine aminopeptidase was controlled by two loci, designated LAP-A and LAP-B. LAP-A was monoallelic, LAP-B consisted of eight alleles. Of the eight LAP-B alleles, LAP-B7 was absent from two of the populations (Bystrzyca and Tomaszow Lubelski) and was infrequent in the other three. LAP-B4 occurred most frequently. Glutamate oxaloacetate transaminase was controlled by three loci, designated GOT-A, GOT-B and GOT-C, consisting of four, 12 and 14 loci respectively. In two of the populations (Kosarzyska and Tomaszow Lubelski) GOT-B5 was the commonest allele, whilst in the other three B1, B8 and B9 were the commonest. The population furthest to the north-east (Tomaszow Lubelski), to which the genetically closest population was that from Kosarzyska in the Carpathians, was the most genetically distant from the others.

140. Mejnartowicz, L. and F. Bergmann. 1975.

Genetic studies of European larch (*Larix decidua* Mill.) employing isoenzyme polymorphisms. Genet. Pol., 16: 29-35.

Using techniques of starch gel zone-electrophoresis, two LAP and one APH (acid phosphatase) zones were found in the haploid endosperm of dormant seeds of larch (*Larix decidua* Mill.). Analysis of the LAP polymorphism reveals two distinct loci with 5 alleles. Analysis of the APH polymorphism reveals one locus with 4 different alleles. The study of the gene frequencies in one population of larch from the Sudety Mts. shows that one locus (LAP-A) has a fixed allele while two other loci (LAP-B, APH) display a definite variability, although with one predominant allele, respectively. The data obtained indicate that at these 3 isoenzyme loci homozygosity prevails in the larch population.

141. Mejnartowicz, L. and F. Bergmann. 1977.

Variation and genetics of ribonucleases and phosphodiesterases in conifer seeds. *Can. J. Bot.* 55: 711-717.

Using techniques of starch gel electrophoresis, isoenzymes of ribonuclease II (RNase, EC 3.1.4.23) and phosphodiesterase (PDase, EC 3.1.4.1) could be identified in endosperm tissue from dry seeds of three conifer species: Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), and Douglas-fir (*Pseudotsuga menziesii*). The RNase patterns mostly exhibited a relatively great number of isoenzyme bands as well as a considerable tree-to-tree variation, whereas the PDase system revealed only one enzyme zone in each of the three seed species. Furthermore, an isoenzyme variation within the PDase zones appeared to be very infrequent and could only be detected in Norway spruce and Douglas-fir. However, the isoenzyme patterns of RNase as well as PDase showed significant differences between the conifer species. The genetic basis of the intraspecific isoenzyme variations could be easily analyzed, since the test material (seed endosperm) represented haploid tissues resulting from macrogametophytes after fertilization. Hence, it was possible to identify three polymorphic RNase gene loci in Douglas-fir seeds, two in Scots pine seeds, and one in Norway spruce seeds. The PDase zone in each conifer species was controlled by one gene locus which revealed allelic forms only in Norway spruce and Douglas-fir seeds.

142. Millar, C.I. 1983.

A steep cline in *Pinus muricata*. *Evolution* 37: 311-319.

Two northern California bishop pine populations differing in stomatal form, monoterpene composition, flowering time, and allozyme frequency are separated by a cline of less than 3 km width. Allelic frequencies at a marker allozyme locus in standing trees changed from an average of 0.97 north of the cline to 0.23 south of the cline. Differences in allelic frequencies between mature trees and embryos are attributed to long distance pollen flow across the cline. Data on wind direction and flowering phenology support this hypothesis. Genetic, ecological, and paleontological evidence suggests that the cline resulted from recent contact of formerly isolated populations.

143. Mitton, J.B. 1983.

Conifers. In: S.D. Tanksley and T.J. Orton (eds.), *Isosymes in Plant Genetics and Breeding, Part B*, pp. 443-472. Elsevier Science Publishers B.V., Amsterdam. (Review)

144. Mitton, J.B. and R. Andalaria. 1981.

Genetic and morphological relationships between blue spruce *Picea pungens*, and Engelmann spruce, *Picea engelmannii*, in the Colorado Front Range. *Can. J. Bot.* 59: 2088-2094.

Nine morphological characters and one enzyme polymorphism were used in a study of hybridization of blue and Engelmann spruce in the Front Range of Colorado. Eighty-eight trees were studied in one pure Engelmann spruce population, one pure blue spruce population, and three sympatric populations along an elevational transect. The morphological data were summarized with principal components analysis, but no clusters were evident in the projections. A protein polymorphism indicated a predominance of different alleles in the two species, and none of the most common expected (hybrid) heterozygotes were seen. The protein data allowed unambiguous assignment of individuals to separate

groups, and subsequently discriminant analysis was performed. The species could be significantly resolved by discriminant analysis of the morphological traits, but a small degree of overlap between species remained.

145. Mitton, J.B. and M.C. Grant. 1980.

Observations on the ecology and evolution of quaking aspen, *Populus tremuloides*, in the Colorado Front Range. *Am. J. Bot.* 67: 202-209.

Three protein polymorphisms and annual width increments measured from cores were used to study the ecology and evolution of quaking aspen, *Populus tremuloides*, Michx., in the Front Range of Colorado. Correlations among elevation, age and diameter of the largest standing ramet, and sex and mean growth rate of 106 clones are reported and discussed. The variance of growth rate was apportioned within and among ramets of a clone; the majority of the variance is environmental, with broad heritability estimated as less than or equal to .32. Analyses of gene and genotypic frequencies of polymorphic proteins revealed little differentiation with elevation, but substantial differences between the sexes. For one of the three pairwise tests, the genotypes are not distributed independently of one another; linkage disequilibrium between a peroxidase locus and phosphohexose isomerase is significantly different from zero. Multiple regression revealed a positive correlation between mean growth rate and degree of heterozygosity.

146. Mitton, J.B.; P. Knowles; K.B. Sturgeon; Y.B. Linhart and M.L. Davis. 1979.

Associations between heterozygosity and growth rate variables in three western forest trees. In: *Proceedings of Symposium on Isozymes of North American Forest Trees and Insects*, July 27, 1979, Berkeley, Calif. Gen. Tech. Rep. PSW-48. Pacific Southwest Forest and Range Exp. Sta., Forest Serv., U.S. Dep. Agric., Berkeley, Calif., pp. 27-34.

For each of three species, quaking aspen, ponderosa pine, and lodgepole pine, we determined

the relationships between a ranking of heterozygosity of individuals and measures of growth rate. Genetic variation was assayed by starch gel electrophoresis of enzymes. Growth rates were characterized by the mean, standard deviation, logarithm of the variance, and coefficient of variation of annual ring widths measured from cores. In aspen, heterozygosity was associated with high mean growth rate, but in ponderosa and lodgepole pines heterozygosity was not associated with mean growth rate. High levels of heterozygosity were associated with high growth variability in aspen and ponderosa pine, and with low growth variability in lodgepole pine.

147. Mitton, J.B.; Linhart, Y.B.; Davis, M.L. and Sturgeon, K.B. 1981.

Estimation of outcrossing in ponderosa pine, *Pinus ponderosa* Laws. from patterns of segregation of protein polymorphisms and from frequencies of albino seedlings. *Silvae Genet.* 30: 117-121.

Six protein polymorphisms and one seedling character, albinism, were used to estimate the level of outcrossing in ponderosa pine, and to investigate several other aspects of the mating system. The six protein polymorphisms give a homogeneous set of estimates for outcrossing with a weighted average of 0.96, and this estimate is in good agreement with estimates based upon seedling albinism and with a previous estimate based upon one protein polymorphism. Row by columns contingency tests of progeny arrays from different seed trees detected no tree-to-tree heterogeneity in the mating system. For two of the six protein polymorphisms, estimates of allelic frequencies in the effective pollen pool differed significantly from the frequencies of adult trees in the population.

148. Mitton, J.B.; Y.B. Linhart; J.L. Hamrick and J.S. Beckman. 1977.

Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. *Theor. Appl. Genet.* 51: 5-13.

Variation of peroxidase enzymes is analyzed both in mature needle tissue and in open-pollinated seedling families of ponderosa pine, *Pinus ponderosa*, and is identified as being controlled by a single Mendelian locus. Variation at this locus, analyzed in 1,386 individuals, is used in the analysis of population differentiation and the mating system. Significant variation of gene frequencies is detected over distances of several hundred meters, and is found to be associated with slopes of different aspects. Ponderosa pine is wind-pollinated, and an analysis of the mating system indicates that the level of outcrossing is greater than 90%. Selection specific for different environments is evidently strong enough to overcome the homogenizing force of migration and produce population fissuring in ponderosa pine.

149. Mitton, J.B.; K.B. Sturgeon and M.L. Davis. 1980.

Genetic differentiation in ponderosa pine along a steep elevational transect. *Silvae Genet.* 29: 100-103.

Data are reported for 7 enzyme polymorphisms in ponderosa pine, *Pinus ponderosa* Laws. var. *scoloporum* Engelm., in 3 ecologically different localities along a steep elevational transect in the Front Range of Colorado. The highest and lowest sites of the transect differ by almost 1000 m. in elevation, but they are only 3 km apart. Heterozygote excesses associated with slope aspect are found at a peroxidase locus, and clinal differentiation is evident at a phosphoglucosmutase locus. Linkage disequilibrium, a measure of non-random association of genotypes at separate loci, is found at each of the localities.

150. Mitton, J.B.; Y.B. Linhart; K.B. Sturgeon and J.L. Hamrick. 1979.

Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *J. Hered.* 70: 86-89.

A method for extraction of enzymes from mature leaves of a diversity of trees is described. The methods of tissue preparation allow samples to be collected at any time of year regardless of

the reproductive state of the plant. We have used this method to detect six enzyme polymorphisms in ponderosa pine and we present evidence to show that the genes coding for these proteins are simple Mendelian loci.

151. Miyazaki, Y. and H. Miyajima. 1981.

Identification of clone varieties in *Cryptomeria japonica* in Kyushu using peroxidase isozyme pattern. In: Proceedings of XVII IUFRO World Congress, Division 2, Congress Group 2 Provenances, Breeding and Genetics. pp. 219-223.

The present study inquires into possibility of applying peroxidase isozyme patterns for identification of various clone varieties of *Cryptomeria japonica* D. Don in Kyushu. Twenty-nine clone varieties collected from six Prefectures in Kyushu were investigated using electrophoresis by starch gel. It was found that among twenty-nine clone varieties, twenty-five were judged from their peroxidase patterns to be different clone varieties from each other, while four were most likely to be composed of two pairs of the same clone. Clone-complex analysis was conducted on four traditional clone groups (strains). It was demonstrated that traditional clone groups of Measa, Hon-sugi and Ayasugi in Kyushu were consisted of multiple clone varieties respectively. It was also suggested that zymography might be useful for certification of progenitor or ancestor tree within a clone.

152. Miyazaki, Y. and K.-I. Sakai. 1968.

Use of zymography for clone identification in forest trees. *Annu. Rep. Nat. Inst. Genet. Jap.* 19: 94-95. [Genetics Abstracts 1970, G6444].

Needle-leaves were collected from different parts of a single tree in 3 trees which were genuine propagules of Kumotooshi, a clone-var of *Cryptomeria japonica*, and peroxidase isozyme patterns studied. The pattern was the same within a tree or clone. Samples were then collected from 51 trees believed to be derivatives from the Kumotooshi cv. Forty-five were genuine and 9 were

false. Thus peroxidase isozyme patterns are useful in determining whether a given tree is a member of a given clone.

153. Moran, G.F.; Bell, J.C. and Hilliker, A.J. 1983.

Greater meiotic recombination in male vs. female gametes in *Pinus radiata*. J. Hered. 74: 62.

Sexually related regional variations in meiotic recombination in *Pinus radiata* is reported. Six different but genetically homogenous clones of *P. radiata* consistently showed higher meiotic recombination in the male gametophyte for the chromosomal interval assayed.

154. Moran, G.F.; Bell, J.C. and A.C. Matheson. 1980.

The genetic structure and levels of inbreeding in a *Pinus radiata* D. Don seed orchard. Silvae Genet. 29: 190-193.

Thirty clones of a seed orchard of *Pinus radiata* were assayed to determine their genotypes at 22 allozyme loci: 12 loci were monomorphic and 10 polymorphic. The average number of alleles per locus (A) was 1.54 and the estimate of total genetic diversity (H) for the seed orchard 0.108. In three annual seed crops of the orchard the allelic frequencies at eight polymorphic loci, both in the pollen and maternal components of the progeny, were in good agreement with the frequencies in the thirty parental clones. From the progeny arrays in the crops the overall rate of outcrossing in the orchard was at least 90%. There were no significant differences between the rates of outcrossing in the three individual annual crops.

155. Moran, G.F. and A.H.D. Brown. 1980.

Temporal heterogeneity of outcrossing rates in alpine ash (*Eucalyptus delegatensis* R.T. Bak.). Theor. Appl. Genet. 57: 101-105.

Three seed crops of a *Eucalyptus delegatensis* population were assayed for their allozyme genotype at three loci to determine estimates of mating system parameters. In the pollen the allelic frequencies at each of the three loci were similar to those in the parents and the progeny. Overall there was a significant amount of inbreeding (23%) in the population. The levels of outcrossing in each crop were significantly different from each other indicating apparent temporal variation in outcrossing rates. The outcrossing rate was greatest in the oldest crop (85%) and lowest in the most recent crop (66%). Mean heterozygosity in the progeny of all three crops was less than the heterozygosity in the parents indicating that selection favors heterozygotes during the life cycle. The implications of a 'balanced' mixed mating system for eucalypt breeding program are discussed.

156. Morris, R.W.; W.B. Critchfield and D.P. Fowler. 1980

The putative Austrain $\times$ red pine hybrid: A test of paternity based on allelic variation at enzyme-specifying loci. Silvae Genet. 29: 93-100.

The authenticity of the most fully documented red pine hybrids, *Pinus nigra* (Austrian pine) $\times$ *P. resinosa* (red pine), was reassessed by a test of paternity based on allelic variation marked by enzyme variants. Seven enzymes were assayed by applying techniques of starch gel electrophoresis to female gametophyte and embryo tissues from seeds of controlled crosses between hybrids. Genetic analyses providing direct evidence of homology between genes from the two parental species indicate gene segregation at five loci, four of which appear to be linked. Data from embryos scored for three of these loci confirm that allele transmission through pollen parallels that found among female gametophytes. With this background, seven alleles at five loci contributed by the pollen parent of the hybrids were identified either by their absence from female gametophytes of the Austrian pine parent or because they are homozygous in some of the hybrids. In addition to these five loci, seven invariant zones of enzyme activity, assumed to be

specified by seven homozygous loci, were assayed. For these loci, alleles contributed by the pollen parent are electrophoretically identical to those of the Austrian pine parent.

Of 14 alleles at 12 loci expected in the pollen parent of the hybrids, female gametophytes of red pine lacked eight. Moreover, red pine embryos with the nominal red pine parent of the hybrids as pollen parent lacked three of four alleles expected at three loci whose pollen transmission was documented in the hybrids. In contrast, a brief survey of female gametophytes of four other *Sylvestres* pines revealed pollen-parent alleles, often in substantial frequencies, at all twelve loci. We conclude that the pollen parent of these hybrids is not red pine, and that pollen contamination by some other *Sylvestres* species accounts for their paternity. It is suggested that other putative hybrids of red pine be accepted with caution.

157. Morris, R.W. and P.T. Spieth. 1978.

Sampling strategies for using female gametophytes to estimate heterozygosity in conifers. *Theor. Appl. Genet.* 51: 217-222.

Unbiased estimators of genotype and allele frequencies and their respective variances are obtained for loci identified by Mendelian segregation in haploid female gametophytes from individual trees. By a minimum sampling variance criterion, the allocation of experimental effort between the number of females gametophytes analysed per tree and the number of trees sampled per population is examined for a fixed total amount of experimental effort. For estimating heterozygosity, the optimum sampling design for many (generally most) cases is three female gametophytes per tree, but may be more than three depending upon the true genotype frequencies in the population. For estimating allele frequencies, the optimum sampling design is one female gametophyte per tree except in cases where a strong negative correlation exists between alleles within genotypes. Guidelines are discussed for determining a suitable number of female gametophytes to be analysed per tree in order to estimate heterozygosity.

158. Muhs, H.-J. 1974.

Distinction of Douglas-fir provenance using peroxidase — isoenzyme — patterns of needles. *Silvae Genet.* 23: 17-76.

(1) 14 provenances from British Columbia, Washington and Oregon collected by the IUFRO (1966/67) were investigated with the help of electrophoresis of peroxidases by using needle extracts of two year old seedlings.

(2) Of 11 isoenzyme bands, 5 bands (no. 2, 3, 8, 9 and 10) showed reproducible results. Their frequencies were estimated with a sample size of 96 per provenance.

(3) The frequencies of three bands (no. 3, 8, and 10) varied strongly between provenances. Bands no. 2 and 9 were found in almost any plant. A trend could not be ascertained for the distribution of the bands.

(4) The provenances were tested by two by two including all characters (bands) with the χ^2 -test. Of 91 provenances pairs, 69 showed significant values at the 5% level, 22 could not be distinguished.

(5) The measure of distinctiveness was defined and a test was developed for it. In 85 provenance pairs the measure of distinctiveness reaches significant values, only 6 pairs were not significant at the 5% level. Therefore the measure of distinctiveness is more reliable than the χ^2 -test with non transformed values.

(6) Isoenzyme frequencies are best qualified for distinction of provenances. Even closely adjacent provenances can be distinguished.

(7) The distribution of the isoenzyme bands was investigated by an analysis of variance. The component of variance "between provenances" was much higher than "within provenances". The F-values for the characters no. 3, 8 and 10 suggest a homogeneous distribution within provenances and heterogeneity between provenances.

159. Muhs, H.-J. 1981

Progress in isozyme studies in Europe since 1976. *In: Proceedings of XVII IUFRO World Congress, Division 2, Congress Group 2 Provenances, Breeding and Genetics.* pp. 205-213.

In Europe a rapid development of isozyme techniques and their application to problems in forest research occurred in the last decade, of which papers and activities especially of the period 1976-1981 are reported here. The activities are grouped into 8 groups covering the topics of methods, inheritance and variation of isozymes, genecological aspects and population structure, linkage studies, measuring genetical parameters, identification, resistance, molecular properties of enzymes, and the interpretation of results. About 40 publications are reviewed, which are dealing with 7 tree species and about 12 enzymes or isoenzyme systems controlled by about 30 loci with more than 2 alleles at each an average. Special emphasis has been focused on the aspects of application in population genetics.

160. Muller, G. 1976.

A simple method of estimating rates of self-fertilization by analysing isozymes in tree seeds. *Silvae Genet.* 25: 15-17

Analyzing isozymes in endosperm and embryo tissue it is possible to estimate rates of self-fertilization of individual trees. Comparing for example leucin-aminopeptidase isozyme patterns of embryo and endosperm tissue of individual seeds of a certain "marker tree", two out of totally four genotypes originating from self-fertilization can be identified definitely. Preliminary results are given within a stand of Norway spruce: the rate of self-fertilization of one spruce tree was estimated to be 14.7%.

161. Muller, G. 1977.

Cross-fertilization in a conifer stand inferred from enzyme gene-markers in seeds. *Silvae Genet.* 26: 223-226.

By analyzing allozymes in viable seeds, cross-fertilization can be studied in conifer stands. As an example, the probabilities are estimated with which the pollen contribution of an individual marker tree in a Scots pine stand can be expected in the seeds of any other tree as a function of the distance between the respective trees:

The genotype at the LAP-B locus of each of 105 neighboring trees is identified by endosperm analysis. The observed genetic structure is presented and compared with the corresponding Hardy Weinberg proportions. The marker tree is the only one carrying the LAP-B₃ allele. To detect its frequency in seed samples of neighboring trees at different distances, embryo analyses are performed. The allelic frequencies at the LAP-B locus of all detected pollen contributions are compared with those of the 105 parental trees. There is no definite indication of genotypic assortative mating at this locus. The frequency of the B₃ marker allele is definitely reduced with increasing distance from the marker tree. By means of the experimental data, the function $f(x) = 20 \cdot e^{-0.38x}$ is derived which represents the estimated probabilities of cross fertilization with respect to the marker tree.

In a previous study of the author, seeds from the same marker tree were identified as originating from self-fertilization. Therefore the mating system of Scots pine can be defined for the present as a system of preferential mating between neighboring trees, including self-fertilization.

162. Muller, G. 1977.

Investigations on the degree of natural self-fertilization in stands of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.). *Silvae Genet.* 26: 207-217.

The detrimental effects resulting from self-fertilization and its significance in forest genetics and forest tree breeding are presented comprehensively.

To identify open pollinated offspring from self-fertilization of individual trees, characters are used, the genetic control of which has been

proven. These are the isoenzymes of the monomeric leucin-aminopeptidase (LAP) in the endosperm and embryo tissue (method (1)) as well as the complete lack of chlorophyll in seedlings (method (2)).

The experimental material originate from continuous parts of stands on which all the seed-bearing trees were harvested separately. The positions of all trees are known. Suitable experimental trees are only those carriers of rare markers whose distances to trees of the same type are large enough to essentially exclude cross-pollination.

To find suitable experimental trees, the LAP-genotypes of all trees are determined by means of endosperm analyses (starch gel zone electrophoresis). If an experimental tree carries one rare allele on the LAP-B-gene locus, then two out of a total of four genotypes resulting from self-fertilization can be identified from its seeds by means of analysis of endosperm and corresponding embryo. If the experimental tree carries two rare alleles, then all the offspring from self-fertilization can be identified in this way.

Applying method (2), a sample of at least 300 seedlings per tree is used to test whether the corresponding mother tree carries the recessive "albino-allele". If a suitable experimental tree is available, then the frequency of the albino-seedlings among the progeny of that tree is determined. Estimating the rates of self-fertilization, it is assumed that these seedlings represent 25% of all the offspring from self-fertilization.

By means of method (1), the rates of self-fertilization of a total of 14 experimental trees from 10 different stands are estimated. Among the identifiable genotypes from self-fertilization of five Norway spruce trees and one Scots pine tree, large deviations, from the expected 1:1 proportion are detected (from 0.6 : 1 to 3.4 : 1). This cannot be explained by gametic selection because of the observed gametic segregations. It can be proven that genotypic selection resulting from archegonial polyembryony can cause distortions within the range from 0.65 : 1 to 1.7 : 1, whereas deviations greater than these are caused by genotypic selection resulting from linkage between marker and lethal genes. From this, two methods are derived to estimate the share of the not identifiable offspring from self-fertilization.

The derived rates of self-fertilization vary from 7.3 to 17.7% in the Norway spruce trees and from 2.9 to 9.6% in the Scots pine trees. Applying method (2), the estimated rates of self-fertilization of a total of 9 experimental trees indicate distinct deviations from the above presented values for the Norway spruce trees. However, a good agreement exists when only the frequency of the homozygous type is taken in consideration. This indicates that in the case of genotypic selection, estimation errors can result from the application of the albino-method. These errors can be avoided only by parallel performance of pollination experiments. However, the isoenzyme-method is independent of such additional experiments.

The average expected rate of self-fertilization derived from the values of the experimental trees carrying isoenzyme-marker genes is estimated to be 11.9% for Norway spruce and 6.2% for Scots pine.

163. Muller-Starck, G. 1979.

Estimates of self- and cross-fertilization in a Scots pine seed orchard. In: Proceedings of the Conference on Biochemical Genetics of Forest Trees, Umea, Sweden, 1978. pp. 170-181.

By analysing enzyme gene-markers in seeds, the probabilities of self- and cross-fertilization are estimated with respect to one specified clone in a Scots pine seed orchard.

The genetic control of the applied enzyme system of leucine aminopeptidase (LAP) is proved by analyses of seeds from controlled crossings. The LAP-B genotypes of the clones are identified by endosperm analysis. One clone carries a unique allele and is thus selected as the marker clone.

The probabilities of self-fertilization are estimated by analysing open-pollinated seeds from each of eight individuals of this clone during three flowering periods. Average period values vary between 0,115 and 0,139. These values have about twice the size of those from analogous studies by the author in Scots pine stands.

Cross-fertilization is studied by detecting the pollen contribution of the marker clone in the seeds of neighboring individuals from different clones. The results from two flowering periods indicate that within the maximum distance of 21 m, cross-fertilization cannot be proved unequivocally to depend on the distance between the respective trees.

The expected average coefficients of inbreeding are computed for the actual seed orchard set as well as for different assumptions concerning number of clones and individuals per clone. Considering constant population size $N=900$, it can be stated, for example, that if the number of clones is increased from the actual value of 36 to 90 clones, the average coefficients of inbreeding can be expected to be reduced in the first generation by about 30%.

164. Muller-Starck, G. 1982.

Reproductive systems in conifer seed orchards. 1. Mating probabilities in a seed orchard of *Pinus sylvestris* L. *Silvae Genet.* 31: 188-197.

Mating probabilities are described in terms of the probabilities of self- and cross-fertilization. They were studied by analysing enzyme gene markers in viable seeds of a Scots pine seed orchard. The genetic control of the applied enzyme system was elucidated. The genotypes of the clones were identified by analysis of endosperm and/or needle tissue. One of the clones carries a unique allele and thus functions as the marker clone.

Probabilities of self-fertilization were estimated by means of identification of those open-pollinated seeds from the marker clone which contain the pollen contribution from this clone. Average values for three flowering periods ranged between 0.115 and 0.139; values for the individual trees of the marker clone varied between 0.063 and 0.267.

Probabilities of cross-fertilization were estimated by detection of the pollen contribution of the marker clone in the seeds of neighboring individuals from other clones. Results from two flowering periods indicated that cross-fertilization

in this case did not depend on the distances between the respective trees.

Mating probabilities are illustrated in the form of three-dimensional plots. A comparison is made between the results of calculations based on hypothetical values and on approximations to the experimental data. Selection against descendants from self-fertilization can thus be documented.

The female and male gametic contributions of the marker clone, including descendants from self-fertilization, were determined in all-orchard seed samples from two flowering periods.

The experimental results were used to calculate the expected average coefficients of inbreeding and kinship in the offspring populations for the actual seed orchard layout and, in general, for varying numbers of clones in seed orchards.

165. Muller-Starck, G. 1982.

Sexually asymmetric fertility selection and partial self-fertilization. 2. Clonal gametic contributions to the offspring of a Scots pine seed orchard. *Silva Fenn.* 16: 99-106.

Enzyme gene markers were applied to identify the gametic contributions of single clones among the open pollinated offspring from a Scots pine (*Pinus sylvestris* L.) seed orchard. Seeds from two flowering periods were studied. The genetic control of the enzyme system GOT was verified by analysis of seeds from controlled crossings. Three clones carried unique alleles and functioned as marker clones. Their gametic contributions were monitored in all-orchard seed probes.

The obtained deviations between the female and male contribution of each clone indicate differential ovule and pollen production. Such occurrence of sexually asymmetric fertility selection was also reflected in the genotypic structure of each offspring population by applying the "multiplicative structure". Individual and clonal self-fertilization were indicated. The obtained results stress the importance of taking into account the effects of sexually asymmetric fertility selection in order to avoid misleading interpretations of genotypic structures among the offspring.

166. Muller-Starck, G. and Huttermann, A. 1981.

Amino-peptidases in seed of *Picea abies* (L.) Karst.: Characterization of leucine aminopeptidase by molecular properties and inhibitors. *Biochem. Genet.* 19: 1247-1259.

By either acrylamide or starch gel electrophoresis of Norway spruce (*Picea abies*) seed extracts, two prominent isoenzyme bands were obtained after staining for leucine aminopeptidase (LAP). These bands were proved to correspond to each other by reelectrophoresis in both gel media. Single endosperm studies with acrylamide gels showed clearly that, in addition to LAP, two bands are expressed after staining for alanine aminopeptidase (AAP) as well. Both the LAP and the AAP activities appeared together as a single peak between catalase and ferritin after gel chromatography on Sepharose. Isoelectric focusing in sucrose gradients proved the two LAP activities to have identical isoelectric points and revealed that LAP, but not AAP, is detectable by standard starch gel electrophoretic procedures. The two LAP bands refer to approximate molecular weights of 71 000 and 131 000, respectively. Disaggregation studies did not conclusively determine whether these two bands represent different enzymes or not. Only inhibitors succeeded in producing a definite differentiation by selective inhibition of one of the two bands. It is concluded that in both gel media the isoenzyme bands reflect the activity of two distinct leucine aminopeptidases.

167. Muller-Starck, G; M. Ziehe and H.H. Hattemer. 1983.

Reproductive systems in conifer seed orchards. 2. Reproductive selection monitored at an LAP gene locus in *Pinus sylvestris* L. *Theor. Appl. Genet.* 65: 309-316.

In a Scots pine seed orchard the genetic structures at an enzyme gene locus active in pine seeds were compared among the parental clones and the all-orchard seeds produced over a period of three years. The genotypes of the seeds were identified as ordered pairs consisting of the female and male contribution. The sexual reproductive function monitored at the studied locus

differed significantly between the two sexes. This fact proves the necessity of taking sexual asymmetry into account in studies of reproductive selection. This is done by comparing the observed genotypic structures among the offspring with the corresponding multiplicative structure expected under random gametic fusion. Additionally, accounting for partial self-fertilization increased the conformity between the observed and the hypothesized model structures. The differences in female and male gametic contributions to the offspring were used to estimate female and male relative fitness components. Significant deviations between the allelic and/or genotypic structures of orchard clones and their seed, and between seed lots collected in different years may reduce the efficiency of realizing breeding gains in seed orchards.

168. Neale, D.B. and W.T. Adams. 1981.

Inheritance of isozyme variants in seed tissues of balsam fir (*Abies balsamea*). *Can. J. Bot.* 59: 1285-1291.

Evidence for the inheritance of isozymes in megagametophyte (1N) and embryo (2N) seed tissues of balsam fir (*Abies balsamea*) (Linn.) Mill.) is presented. Segregation of isozymes in megagametophytes of heterozygous mother trees and comparison of banding patterns with those of other conifers indicate that a minimum of 14 loci code isozymes in the enzyme systems studied. Isozymes coded by 13 of these loci are detectable in both seed tissues; one locus is apparently active only in haploid tissue. Two loci segregation data were available for 20 of the 28 possible pairwise combinations of eight polymorphic loci. Chi-square tests of independence revealed that five of these pairs are linked. Genes similar to those in two of the pairs are linked in other *Pinus* species.

169. Nikolic, D.J. and Bergmann, F. 1974.

Genetic variation of leucine aminopeptidase [LAP] isoenzymes in seeds of *Pinus nigra* Arn. *Genetika*, 6: 361-365. [Forestry Abstracts 37, 830].

Female gametophyte endosperm from the mature seed of *P. nigra* from trees in different localities in Yugoslavia was analyzed by starch gel electrophoresis. Two distinct polymorphic LAP zones were found; these could be shown to be controlled by two separate gene loci with five alleles, two of which represent so-called zero-alleles (recessive type).

170. Nikolic, D.J. and N. Tucic. 1983.

Clinal variation of null alleles in European black pine (*Pinus nigra* Arn.). Genetika, 15: 105-114.

Electrophoretic analyses of isoenzyme variation have shown that the average frequencies of null alleles at four loci of *Pinus nigra* exhibit clinal variation with respect of latitude. Spaerman's correlation coefficient calculated between the average frequencies of null alleles in each of 28 local populations of black pine and their latitudinal positions in the Mediterranean area analyzed is negative and highly significant ($r_s = -0.573$). The present results are in good accordance with the hypothesis that null alleles have an important role in the maintenance of genetic variability of black pine populations.

171. Nikolic, D.J. and N. Tucic. 1983.

Isoenzyme variation within and among populations of European black pine (*Pinus nigra* Arnold). Silva Genet. 32: 80-89.

Genetic variation in 28 natural populations of European black pine (*Pinus nigra* Arnold) is studied at the enzyme level. Using techniques of gel electrophoresis, we have characterized the isoenzyme patterns of esterase (Est), acid phosphatase (Acph) and leucine aminopeptidase (Lap) in dormant seeds. The samples examined are from 19 localities in Yugoslavia and nine localities in other Mediterranean countries.

The study concerns the organization of isoenzyme variation throughout the following subspecies of *P. nigra* (classification based on Vidakovic 1974): ssp. *austriaca* (7 populations), *dalmatica* (5 populations), *pallasiana* (4 populations),

gocensis (4 populations), *marocana*, *Fenzlii*, *corsicana*, *laricio*, *Villette Barrea* (all with one population) and two populations from Cyprus.

The mean value for all samples of the average expected heterozygosity over four loci is 27.2%. The average number of alleles detected per locus is 3.02.

Analysis of population subdivision using Nei's (1973) statistics indicates that heterogeneity within populations is higher than heterogeneity between populations or subspecies.

The genetic distances (Nei 1972) associated with various stages of evolutionary divergence are calculated. The average genetic distance for all 378 pairwise population comparisons of *P. nigra* is 0.069 ± 0.004 . The average genetic distance between pairs of recognized subspecies ranges from 0.017 (e.g., for ssp. *Villette Barrea* and *Fenzlii*) to 0.389 (for ssp. *marocana* and *laricio*) with the mean value for all 55 pairwise comparisons of 0.111 ± 0.01 .

Differences in allelic frequencies are statistically significant among the localities examined, but fail to show any decisive geographic patterning. Roylance *et al.* (1975) tests and autocorrelation analyses, both based upon Gabriel connected-graphs for 19 localities from Yugoslavia, are used to determine whether the distribution of gene frequencies depart from a random pattern. Significant deviations are absent in Yugoslavian localities of *P. nigra*. Different models of population differentiation are discussed.

172. Nordheim, E.V.; D.M. O'Malley and R.P. Guries. 1983.

Estimation of recombination frequency in genetic linkage studies. Theor. Appl. Genet. 66: 313-321.

A binomial-like model is developed that may be used in genetic linkage studies when data are generated by a testcross with parental phase unknown. Four methods of estimation for the recombination frequency are compared for data from a single group and also from several groups; these methods are maximum likelihood, two Bayesian procedures, and an *ad hoc* technique.

The Bayes estimator using a noninformative prior usually has a lower mean squared error than the other estimators and because of this it is the recommended estimator. This estimator appears particularly useful for estimation of recombination frequencies indicative of weak linkage from samples of moderate size. Interval estimates corresponding to this estimator can be obtained numerically by discretizing the posterior distribution, thereby providing researchers with a range of plausible recombination values. Data from a linkage study on pitch pine are used as an example.

173. Ohlsson, P.-I. 1979.

On the extraction of isoperoxidases from needles of *Pinus sylvestris*. In: Proceedings of the Conference on Biochemical Genetics of Forest Trees, Umea, Sweden, 1978. pp. 25-28.

A procedure to obtain stable isoperoxidase extract from needles of *Pinus sylvestris* is described. Available methods have been critically examined and possible sources of errors are discussed.

174. Olsson, U. 1975.

Peroxydase isozymes in *Quercus petraea* and *Quercus robur*. Bot. Not. 128: 408-411. [Genetics Abstracts G8, 8764].

An investigation of the occurrence of multiple molecular forms of peroxydases in the leaf tissues of *Q. petraea* and *Q. robur* has been carried out. In all, 11 different isozymes have been found. These are classified into 4 groups, one of which is only found in *Q. petraea* and introgressive populations. However, one of the *petraea* populations examined also lacks this group of peroxydases. The possibility of the occurrence of introgressive individuals not revealed by morphological analysis is discussed. The intraspecific variation of the zymograms is great, indicating the genetic heterogeneity of oak populations.

175. O'Malley, D.M.; F.W. Allendorf and G.M. Blake. 1979.

Inheritance of isozyme variation and heterozygosity in *Pinus ponderosa*. Biochem. Genet. 17: 233-250.

Techniques are presented to detect 23 isozyme loci in the long-lived perennial plant, ponderosa pine. Meiotically derived megagametophyte from seeds is used to examine directly the segregation of allelic variants. Approximately seven seeds were initially examined for 12 enzymes from each of 47 trees from ten stands throughout the northern Rocky Mountain region. Additional seeds were also examined from selected families to confirm the inheritance of observed electrophoretic variants at 13 polymorphic loci and to estimate linkage relationships. Significant non-random segregation was consistently detected for three pairs of loci: ADH-1: AAT-2, ADH-1: PGI-1, and LAP-2: 6PG-1. Preliminary estimates of population parameters reveal a relatively high average heterozygosity ($H=0.123$). This is partitioned into a high amount of genetic variation within local stands, with only approximately 12% of the total heterozygosity resulting from genic differences between stands.

176. Park, Y.G. 1972.

Gene flow in natural forest of *Cryptomeria japonica* by means of isozyme polymorphisms. Research Report of the Institute of Forest Genetics, Korea, 9: 77-85. [Forestry Abstract 34, 6761].

In this study of genetic variation in natural forests, pollen samples were collected from ca. 50 trees from each of three sub-populations of *C. japonica* in Yanase natural forest, Shikoku, Japan. Of the three sub-populations, (1) and (2) were ca. 760 m apart and (3) was ca. 1300 m from (1) and (2). The samples were stored in a deep freezer at -20°C , and later used in an electrophoretic analysis. The total number of isoperoxidase bands was 36 and the mean number of bands in (1), (2) and (3) was 11.68, 13.73 and 12.53 respectively. The variation in frequency of occurrence of each band among the sub-populations was statistically significant. This result is discussed in relation to other studies on pollen

dispersal that have given rise to two views on the possibility of gene flow within a natural forest: some investigators consider that the distance of pollen dispersal is short and that gene flow among sub-population seldom occurs, while others consider that pollen travels long distances and that no effective isolation is possible within a forest, any regional genetic differentiation that may have occurred being attributable not to gene flow but to regional selection pressure. The study reported lends support to the former view.

177. Park, Y.G. and W.H. Son. 1979.

Silvicultural and genetic studies on isozyme patterns in forest trees. Inheritance of leucine aminopeptidase and peroxidase isozymes in *Pinus taeda* × *P. rigida* and *P. densiflora*. J. Kor. For. Soc. 43: 39-50. [Forestry Abstract 42, 4044].

Hybrids from pollinating *P. taeda* trees with *P. rigida* pollen were studied as well as *P. densiflora* hybrids. Leucine aminopeptidases (LAP) were shown to be controlled by three alleles at the LAP-A locus and two alleles at the unlinked LAP-B locus. In the *P. taeda* × *P. rigida* hybrids, peroxidases (Px) were controlled by genes at three loci, Px-A, Px-B and Px-C. There may be two alleles at the Px-A locus.

178. Perry, T.O. 1963.

Differences in protein constituents in dormant and vegetative plant tissue [*Spirodela polyrrhiza* and *Pinus thunbergii*]. Aibs Bulletin, 13: 73-75. [A note].

179. Perry, T.O. 1971.

Seasonal and genetic differences in fats, phenols, isoenzymes, and pigments of red maple. For. Sci. 17: 209-212.

Racial differences in dormancy patterns, cold tolerance, and chilling requirements are accompanied by differences in fat, phenolic, isozyme, and pigment content. These differences

are most marked in November and disappear with the progress of the winter season.

180. Phillips, M.A. and A.H.D. Brown. 1977.

Mating system and hybridity in *Eucalyptus pauciflora*. Aust. J. Biol. Sci. 30: 337-344.

Allozyme polymorphisms at four loci expressed in seeds, and three other loci expressed in seedlings, were used to determine the outcrossing rate in three natural subalpine populations of snow gum (*E. pauciflora*). Based on the seed loci data, an estimated 37% of seed was derived from self-fertilization and 65% from random outcrossing. In the most elevated population the estimate after germination was similar. However, at lower elevations the frequency of effective self-fertilization estimated at the seedling stage was only 16%. The less elevated populations also showed a greater average heterozygosity and a larger increase in heterozygosity in the adult over the progeny stages. Heterosis apparently operated differentially in these populations — it was more intense at the lower altitudes. Selection in favour of outcrossed individuals may be an important factor in checking the spread through the population of genes which promote self-fertilization, and which would otherwise enjoy an evolutionary advantage.

181. Pitel, J.A. and Durzan, D.J. 1976.

Nuclear proteins of dry and germinating conifer seeds. In: Proceedings of the 12th Lake State Forest Tree Improvement Conference, August 1975. U.S.D.A. Forest Service General Technical Report, North Central Forest Experiment Station, No. NC-26, pp. 147-157. [Forestry Abstracts 43, 317].

Polyacrylamide gel electrophoresis was used to determine patterns of nuclear, histone, and non-histone chromosomal protein fractions in *Pinus banksiana* seeds. The method is considered suitable for studying the genetics of nuclear protein metabolism for tree improvement programs.

182. Polozova, L.Ya. and V.A. Dukharev. 1978.

Variation in the esterase enzymes in a two-storeyed population of Scots pine. *Lesovedenise*, 5: 30-35. [Forestry Abstracts 40, 1407].

A study was made of the genetic polymorphism of a two-storeyed stand of *Pinus sylvestris* in the Moscow region. The first storey consisted of trees of average age 140 years, and the second of trees aged 45 years. The esterase isozymes of the needles and seeds were investigated using the method of Feret. Four spatially distinguishable regions of enzyme activity were observed on the zymograms of the esterases of the seed endosperms and needles, and these were designated A, B, C and D, according to their mobility in the electrical field. Seven esterase phenotypes were represented in the ratio 1:2:3:3:3:2:1 in the first and second storeys of the stand. The trees in the two storeys had similar morphological features. The coefficient of variation in annual-ring width decreased with increasing number of isozymes in the esterase spectrum of trees in both storeys. No correlation was found between the number of esterase isozymes and the seed wt., seed colour, cone, number of seeds per cone, and needle length.

183. Polozova, L.Ya.; L.F. Praudin and V.A. Dukharev. 1978.

Biochemical polymorphism in two-storeyed populations of *Pinus sylvestris*. *Doklady Akademii Nauk SSSR*. 243: 778-781. [Forestry Abstracts 41, 1983].

A study of a population from Zvenigorod in Moscow province, in which trees of the first storey were 140-150 yr old and those of the second storey 35-45 yr, and a population from the Mari ASSR, in which the trees of the first storey were 170-180 yr and those of the second storey 70-80 yr old. An electrophoretic analysis was made of 4 loci controlling acid phosphatase, 4 controlling alkaline phosphatase, 5 controlling esterase and 2 controlling leucine aminopeptidase in the seeds (haploid) and needles (diploid); 10 loci were polymorphic. Data are tabulated on the frequency of occurrence of alleles of the loci and

on the distribution of genotypes in the trees studied: heterozygous loci predominated. Intrapopulation heterozygosis tended to increase with age.

184. Rasmuson, B. and D. Rudin. 1971.

Variations in esterase zymogram patterns in needles of *Pinus sylvestris* from provenances in northern Sweden. *Silvae Genet.* 20: 39-41. [Preliminary report].

185. Rasmuson, M. 1979.

Interaction between loci-models and observations. In: *Proceedings of the Conference on Biochemical Genetics of Forest Trees*, Umea, Sweden 1978, pp. 62-72.

Most population genetic models use the gene as unit of selection and disregard the importance of linkage and epistasis. It is only since the 1960s that it has been appreciated that so called *linkage disequilibrium* may occur in stable situations. At equilibrium the gamete frequency for a pair of loci is expected to be the product of the gene frequencies, $\bar{g}_{AB} = P_A P_B$, etc. Disequilibrium is therefore measured as $D = \bar{g}_{AB} - P_A P_B$. The D parameter is of special interest since varying assumptions result in different predictions about its value. It is thus of importance to study this parameter in natural populations, and allozyme analysis has made this possible in many organisms.

In four populations of *Picea abies* seven polymorphic allozyme loci were tested for linkage disequilibrium in pairwise combinations. 9 cases among 84 showed a significant non-zero D-value and in the pooled material there were four significant deviations among 21 combinations. One of these occurred between two closely linked loci. In seven populations of *Pinus sylvestris* pairwise combinations of three polymorphic allozyme loci yielded only one significant non-zero value of D.

186. Roberds, J.H. and M.T. Coukle. 1984.

Genetic structure in loblolly pine stands: allozyme variation in parents and progeny. *For. Sci.* 30: 319-329.

Genetic population structure in two adjacent old-field stands of loblolly pine was studied by analyzing allele frequencies for eight polymorphic isozyme loci. The stands represented different generations with trees in older stands being parents of trees in the younger one. The parental stand consisted of three fairly distinct age classes which differed significantly in allele frequencies for four of the allozyme loci, yet no differences were found among the spatial divisions studied. Within the progeny stands, no genetic differentiation was detected with respect to either age class or spatial grouping. Although allele frequencies did not differ between the two stands, genetic population structure was not the same, demonstrating that local genetic structure can differ between successive generations and that varying structures can exist in localized regions. Evidence suggesting low level of inbreeding was found in both stands.

187. Rudin, D. 1974.

Gene and genotype frequencies in Swedish Scots pine populations - A study by the aid of the isozyme technique. *In: Proceedings, Joint IUFRO Meeting, S.02.04.1-3, Stockholm, 1974, pp. 465-467. [Special report].*

188. Rudin, D. 1975.

Inheritance of glutamate-oxalate-transaminases (GOT) from needles and endosperms of *Pinus sylvestris*. *Hereditas*, 80: 296-300 [Brief report].

189. Rudin, D. 1976.

Biochemical genetics and selection application of isozymes in tree breeding. *In: Proceedings of the IUFRO Joint Meeting of Working Parties on Population and Ecologi-*

cal Genetics, Breeding Theory, Biochemical Genetics and Progeny Testing, Bordeaux 1976, pp. 145-164.

Applications of isozyme technique are discussed in the light of the present state of isozyme investigations in forest trees and some results from other plant species. A desirable number of polymorphic isozyme loci optimizing the usefulness of this method ought to exceed the chromosome number in the gametes. Linkage studies are necessary to establish the distribution of available loci over the genome. The seven main steps necessary to bring a new enzyme system into a useful position are pointed out.

Experience with studies of *Avena*, *Bromus* and *Lolium* indicates that there are good possibilities for study of mating systems, response to different selection intensities and multilocus organization.

The use of isozyme marker genes in different breeding strategies is discussed from the forest tree breeder's point of view. These strategies are: mass selection, hybrid varieties and recurrent selection. Some studies in seed orchards using isozyme marker genes are also suggested. The conclusion which could be drawn from this literature review is that the opportunities to use isozyme marker genes for forest tree breeding purposes are fairly good and isozymes will be an indispensable tool in the future.

190. Rudin, D. 1977.

Leucine-amino-peptidases (LAP) from needles and macrogametophytes of *Pinus sylvestris* L. Inheritance of allozymes. *Hereditas*, 85: 219-226.

The mode of inheritance of two LAP allozyme loci in *Pinus sylvestris* is reported. For each locus the bands are clearly separated into two regions in the gels after staining for LAP. The allozymes appear like monomeric products, i.e., two bands in a heterozygote. The analyses indicate four alleles for both loci, LAP-A and LAP-B.

Among a total of 140 trees 18 were considered to be heterozygous for locus LAP-A and 23 heterozygous for locus LAP-B on the basis of endosperm or/and needle analyses. Of these, six for

the former and eleven from the latter group were chosen for closer studies. In order to study the postulated alleles, suggested on this basis of the banding pattern, 12 crosses were analyzed.

The total results indicate that in most cases the gametic segregations of the two loci LAP-A and LAP-B are very close to 1:1 in female as well as male gametes. For both loci, however, there are exceptions. The genetical mechanisms causing these exceptions are discussed.

191. Rudin, D. and I. Ekberg. 1978.

Linkage studies in *Pinus sylvestris* L. — using macrogametophyte allozymes. *Silvae Genet.* 27: 1-12.

Linkage studies between 12 allozyme (allelic isozyme) loci from macrogametophytes of *Pinus sylvestris* are reported. Seeds for isozyme analyses were collected mainly from single trees in one seed tree stand in northern Sweden, but trees from other parts of Sweden were also included. The following enzyme systems and numbers of loci are represented: Acid phosphatases (PHOS) — two loci, alcohol dehydrogenases (ADH) — two loci, esterases (EST) — one locus, glutamate oxalate transaminases (GOT) — three loci, leucine aminopeptidases (LAP) — two loci and malate dehydrogenases (MDH) — two loci. Four of these ADH-MA and — MB — LAP-B — GOT-B are associated in a well established linkage group I. Three single trees show linkage between ADH-MB in group I and GOT-A, LAP-A and MDH-MB. Possible linkage may also exist between MDH-MB — PHOS-MA — GOT-MC. This linkage group is indicated by data from five trees. Estimations of map distances have been made according to Kosambi (1944) and Jensen and Helms Jørgensen (1975). Map distances between ADH-MA and -MB were found to be zero but the distance between these and LAP-B were estimated to be 32.8 ± 3.1 cM (centi Morgan) and between LAP-B — GOT-B 15.5 ± 1.4 cM. A positive interference within group I was found in two triple heterozygotes.

192. Rudin, D. and Ekberg, I. 1982.

Genetic structure of open-pollinated progenies from a seed orchard of *Pinus sylvestris*. *Silva Fenn.*, 16: 87-93.

Five clones carrying isozyme marker alleles in the glutamate-oxalatetransaminase and leucine-amino-peptidase loci were chosen in a seed orchard of *Pinus sylvestris* in central Sweden. In three of them, cones were collected from two vertical sections and in the two others from three sections, one top section two meters above ground, one inner section and one peripheral section below the two meter level. Cones from the closest four neighboring grafts are a distance of five meters as well as from four grafts at a distance of 14 meters from the marker grafts were also included in the collection program. The frequencies of marker alleles found in the surrounding grafts reached one to three percent which agrees with the expected, following panmixis. Based on seven different estimates from heterozygous marker loci the mean selfing frequency amounted to 16 percent. The lower inner sectors of the crowns have a selfing frequency of seven percent. These selfing frequencies are surprisingly high. The relevance and significance of these results are discussed.

193. Rudin, D.; G. Eriksson and M. Rasmussen. 1977.

Inbreeding in a seed tree stand in northern Sweden. A study by the aid of the isozyme technique. *In: The Isozyme Technique — A short-cut to the genes of our forest trees?* Umea, Sweden. 45 p.

Isozyme analyses were done in order to study inbreeding at two levels, namely, coancestry of adult trees and the occurrence of selfing judged from their progeny. For this purpose 57 widely spaced seed trees were studied in a seed tree stand in northern Sweden. The results indicate a certain degree of coancestry based on the spatial distribution of rare alleles. The selfing frequency was studied in embryos from seven trees and two-year-old plants from four trees. The average selfing frequency was 24 and 17 percent, respectively. The causes and implications for these high frequencies are discussed.

194. Rudin, D.; G. Eriksson; I. Ekberg and M. Rasmuson. 1974.

Studies of allele frequencies and inbreeding in Scots pine populations by the aid of the isozyme technique. *Silvae Genet.* 23: 10-13.

The allele frequencies in three isozyme loci of three Swedish pine populations were estimated. The populations were tested with respect to homogeneity. The tests revealed heterogeneity of allele frequencies for the EST-B locus (5 percent significance) and for the GOT-B locus (0.1 percent significance) whereas high *p* value for homogeneity was obtained for the LAP-B locus. The populations were tested for agreement with expected genotype frequencies following random mating. A general excess of homozygotes was found, but the deviations were significant only for the EST-B locus of the Gardstjärn population (lat. 65°29'; alt. 400 m). The calculated inbreeding coefficient for this population for the EST-B locus was 0.33.

195. Rudin, D. and D. Lingren. 1977.

Isozyme studies in seed orchards. *Studia Forestalia Suecica*, Nr 139, 23 p.

Some applications of the isozyme technique for studies in seed orchards are discussed. The basis for these studies is the opportunity to use clones in the seed orchard which are isozymetically distinct from the others. If a clone in one locus carries one or two alleles unique or rare to the orchard, it may then be possible to trace the contribution of this particular clone to the seed formation. The use of this method in studies on the net effect of open pollination in a seed orchard is pointed out.

In the absence of such unique alleles for the estimation of the pollination situation, another method is suggested. This method can be made use of by collecting open-pollinated seeds from a clone and counting plants from these which, by means of the isozyme pattern, show a documented cross-pollination. In this way it is possible to obtain information on the occurrence of selfing.

Checking of the isolation technique for controlled crosses is also possible with the isozyme technique.

The theoretical opportunities for future studies of correlations between economically important traits and allozymes (allelic isozymes) are discussed briefly.

The theoretical possibilities are partly illustrated by results obtained from Swedish Scots pine seed orchards. No indication of deviation from random mating were found. The frequency of plants originating from selfing following open pollination was indicated to be in the range of 2-5 percent. Such plants seemed to be more common in the lower than in the upper part of the crowns of the ramets. The relevance of checking controlled crosses is demonstrated by means of an applied example.

196. Rudin, D. and K. Lundkvist. 1977.

Forest isozyme studies in Umea, Sweden. In: EEC Symposium on Forest Tree Biochemistry, Commission of the European Communities, Brussels, 25-27 January, 1977. pp. 133-150.

The forest isozyme programme of the Umea group, Sweden, is presented. Some important prestudies before the use of needles as a source of information are suggested. Studies on the mode of the inheritance both for needles and macrogametophytes (edosperms) are described for the two species of our interest, namely *Picea abies* and *Pinus sylvestris*. Linkage studies between 13 allozyme (allelic isozymes) loci of *Pinus sylvestris* are announced.

Variation within populations is discussed in terms of "average heterozygosity" according to Nei and Roychoudhury (1974). Some recent calculations of this measure comprising 11 Swedish stands of *Picea abies* are performed. Among other forces which are creating genotypes in our stands, pollen distribution and gamete selection are illustrated by some preliminary results from one stand of *Pinus sylvestris* in northern Sweden. Inter-populations variation is estimated as clinal variation of gene- and genotype frequencies, adaptive significance and genetic distances. Calculations according to these measures based on results from Swedish stands of *Picea abies* and *Pinus sylvestris* are presented. Good possibilities to distinguish introduced seed sources are demonstrated.

Some applications of allozyme studies in seed orchards are pointed out. The bases for these applications are mainly the use of rare or unique allozymes. Recent studies evaluating the net effect of open pollination in a Swedish seed orchard are briefly referred to.

Finally, a brief discussion concerning future studies of correlations between phenotypical traits and allozymes is presented.

197. Rudin, D. and B. Rasmuson. 1973.

Genetic variation in esterases from needles of *Pinus sylvestris* L. *Hereditas*, 73: 89-98.

Starch gel electrophoresis was used to determine the genetical variation of esterases in needles from Scots pine, *Pinus sylvestris*.

Eight mother trees from the middle part of Sweden were investigated. From 7 to 21 selfed progeny from each mother tree and from 17 to 47 progeny from certain crosses between these mother trees were investigated. The results demonstrate the presence of at least 6 independent two-allele systems. Three could be genetically verified through available crosses.

198. Rudin, D. and P.M.A. Tigerstedt. 1979.

Genetic structure of a naturally regenerated stand of *Pinus sylvestris* L. at Oulanka in Finland — A study by the aid of isozyme markers. *Aquilo Ser. Zool.* 20: 92-93. [Preliminary report].

199. Sakai, K.-I. 1971.

Genetic differentiation in a natural population of forest tree species. *SABRAO Newslett.* 3: 71-72. [Genetics Abstracts 1971, G7664].

The techniques used for the genetic analysis among sub-populations of *Thujopsis dolabrata* and *Cryptomeria japonica* within a forest were the study of isozyme patterns of peroxidase and mor-

phological characters. The study of *T. dolabrata* revealed that 2 populations separated by a 10 kilometer wide waterway were genetically distinct from each other. Investigations of 3 sub-populations of *C. japonica* which were not isolated from each other showed that they were distinct from each other. It was concluded that a natural population of wind-pollinated tree spp is not homogeneous genetically, and that the distance of pollen dispersal is short and gene flow in a forest is restricted.

200. Sakai, K.-I. 1981.

Isozyme studies in natural stands of *Cryptomeria japonica*. In: *Proceedings of XVII IUFRO World Congress, Division 2, Congress Group 2 Provenances, Breeding and Genetics*, pp. 205-213.

This paper describes results of the study of breeding structure in five natural populations of *Cryptomeria japonica* by the aid of isoperoxidase zymograms developed on starch gels. Based upon similarities in isoperoxidase patterns among individual trees within populations, it has been inferred that propagation and dispersal of juvenile trees in a stand started from trees growing in around the mid-slope instead of from those growing in ridge- or foot-side of the slope.

A few further facts about propagation of *Cryptomeria* trees have also been detected. The concern dispersal of offspring on the slope, percentage of effectively propagating trees in a stand and the relation between successful propagation and tree size in *Cryptomeria*.

201. Sakai, K.-I.; S. Hayashi and S. Iyama. 1974.

Genetic studies in natural populations of *Pinus*. I. Genetic variability in local populations from several prefectures. *Memoirs of the Faculty of Agriculture, Kagoshima University*, 10: 37-49. [Forestry Abstracts 36, 81].

Needles were collected from a total of 903 individual trees of *Pinus thunbergii* growing at various

coastal sites in six prefectures from SW Kyushu to NE Honshu, and were crushed to provide sap for a study of their isoperoxidase content by starch-gel electrophoresis. The pattern of isoperoxidase bands varied both between trees within each population and between the different populations. The variation between populations included differences in (a) the total number of isoperoxidase bands per tree and (b) the frequency of occurrence of several of these bands. The variations were quite distinct between prefectures and showed a tendency for both (a) and (b) to increase from NE Honshu to SW Kyushu. A study of correlations between (a) and latitude or longitude indicated that this clinal variation was correlated with longitude, and it is concluded that such variation can probably be attributed to random genetic drift during the propagation and spread of the species.

202. Sakai, K.-I.; S. Hayashi and K. Tomita. 1975.

Genetics in natural stands of forest trees. SABRAO J. 7: 137-146. [Genetics Abstracts G8, 5959].

The authors have performed genetic analyses on 2 species of conifers, *Pinus thunbergii* and *Cryptomeria japonica*, which are in danger of extinction because of forest clearing or drastic insect injury. Exterior and interior characters and isoperoxide variation in the needles were investigated on the assumption that they were generally not susceptible to outside influences. Geographic descriptions, correlation coefficients between needle characters and geographic parameters and geographic variation, based on variance analysis and correlation calculations, of *P. thunbergii* populations and intra-population variability, expressed in terms of standard deviation or coefficient of variability, of *C. japonica* are presented.

203. Sakai, K.-I.; S. Iyama; Y. Miyazaki and S. Iwagami. 1972.

Genetic studies in natural populations of forest trees. In: Proceedings of the Joint Symposia for Forest Tree Breeding of Genetics Subject Group, IUFRO, and Section 5, Forest

Forest Trees, SABRAO, Tokyo, Japan, Government Forest Experiment Station of Japan. A-4(I), 1-13.

By means of the electrophoretic comparison of peroxidase components among individual trees vegetatively propagated clones were located in natural populations of *Cryptomeria japonica*. Three parameters, i.e., average clone size, cloning ability and clone ratio have been worked out in order to define natural forests with regard to the capacity of asexual propagation.

Further use of zymograms has been made for detecting seed-propagated families in natural forests of *Thujopsis dolabrata*. The basis for this work is that genetically related individuals are more alike with each other than non-related trees in the pattern of isoperoxidases. Genetic parameters for a few needle characters have been investigated on the basis of the detected families by the analysis of variance. It has been noticed that genetic interpretation of the variance components usually put by animal geneticists is unwarrantable for natural forests of *Thujopsis dolabrata* probably because of occurrence of self-fertilization or inbreeding there. A parameter, the degree of family likeness, has been computed.

204. Sakai, K.-I. and Y. Miyazaki. 1972.

Genetic studies in natural populations of forest trees. II. Family analysis: A new method for quantitative genetic studies. Silvae Genet. 21: 149-154.

1. Two natural forests of *Thujopsis dolabrata* were investigated for the electrophoretic peroxidase variation.

2. It was found that trees alike in the isozyme pattern were growing within a circular area of about 20 or 25 meter radius. It is considered that genetically related trees are growing in proximity and are not scattered.

3. Variation in a few characters among trees of similar isozyme patterns showed that the variance of the characters among those trees was so small that they had to be considered to be members of the same family.

4. There is an indication that trees in a natural forest of *Thujopsis dolabrata* are more homozygous than expected from random mating.

5. The technique of "family analysis" described in this paper may enable us to carry out a genetic analysis of physiological and quantitative characters in a natural forest.

205. Sakai, K.-I.; Y. Miyazaki and T. Matsuura. 1971.

Genetics studies in natural populations of forest trees. I. Genetic variability on the enzymatic level in natural forests of *Thujopsis dolabrata*. *Silvae Genet.* 20: 168-173.

The present paper mainly reports our finding of genetic variation in leaf peroxidase components of *Thujopsis dolabrata* as demonstrated by starch-gel electrophoresis. At first, as many bands as were apparently different were counted. The number attained was 53. Then, by measuring intra-tree correlation among the bands, the possibly non-existent bands were discarded from the count. The final bands identified were 42.

Two natural forests of *Thujopsis dolabrata* which are separated by straits 10 kilometers wide were examined to find out if they were statistically different with regard to the occurrence of those 42 bands. Several leaf characters were also compared between the two forests. It has been concluded from this study that the two natural forests were genetically differentiated from each other.

206. Sakai, K.-I. and Y.-G. Park. 1971.

Genetic studies in natural populations of forest trees. III. Genetic differentiation within a forest of *Cryptomeria japonica*. *Theor. Appl. Genet.* 51: 13-17.

The present study deals with the genetic differentiation within a natural forest of *Cryptomeria japonica*. Needle leaves were collected from approximately 50 trees in each of three sites, called subpopulations I, II and III located at the apices of a triangle. The distances from each other among these three subpopulations were 760 m or

1 300 m. It was found, by an electrophoretic study of peroxidase, that the mean number of isozyme bands exhibited per individual trees was 11.68 in I, 13.73 in II and 12.53 in III. The t-test revealed that the differences between I and II and between II and III were statistically significant. The frequency of occurrence of each band was compared among the subpopulations. It was found that the variation among the three subpopulations was statistically significant. Not a few papers dealing with the study of pollen dispersal in coniferous trees have been published so far. Some of the authors consider that the distance of pollen dispersal is short and gene flow among subpopulations within a forest seldom occurs, while others are of the opinion that pollen travels long distances and no effective isolation is possible within a forest. Findings from the present study support the former view. They show that subpopulations within a forest of *Cryptomeria japonica* are genetically differentiated from each other, i.e., Sewall Wright's neighborhood size is small in *Cryptomeria japonica*.

207. Scholz, F. 1973.

Biochemical methods in forest genetics. *Forstarchiv*, 44: 171-174.

Following the series gene → enzyme → metabolic process → trait it is shown how genetic problems can be worked on with the aid of biochemical methods at the stage of the trait by gas chromatography of terpenes to identify hybrids of parents which are difficult to differentiate morphologically.

The metabolism by the determination of the buffer capacity of pine needles for an indirect diagnosis of the resistance against needle cast of pine (*Lophodermium pinastri*). The enzyme by determining the variability of *Lophodermium pinastri* with the aid of isoenzymes for estimating the success of resistance breeding and by enzymatic tests for susceptibility of enzymes to immission with the purpose of an early diagnosis of the immission resistance of forest trees.

The gene by testing the composition of DNA to find out the relationship between species and their phylogenetic position.

208. Shaw, D.V. and R.W. Allard. 1979.

Analysis of mating system parameters and population structure in Douglas-fir using single-locus and multilocus methods. *In*: Proceedings of Symposium on Isozymes of North American Forest Trees and Insects, July 27, 1979, Berkeley, Calif. Gen. Tech. Rep. PSW-48. Pacific Southwest Forest and Range Exp. Sta., Forest Serv., U.S. Dep. Agric., Berkeley, Calif., pp 18-22.

Two methods of estimating the proportion of self-fertilization as opposed to outcrossing in plant populations are described. The first method makes use of marker loci one at a time; the second method makes use of multiple marker loci simultaneously. Comparisons of the estimates of proportions of selfing and outcrossing obtained using the two methods are shown to yield additional information about inbreeding and the genetic structure of populations. Single-locus estimates of the proportion of selfing in Douglas-fir were heterogeneous over marker loci and lower than estimates obtained with the multilocus estimator. Family structure and/or microhabitat selection were discussed as likely causes of the biased estimates of the proportion of selfing obtained from the single-locus estimation procedure.

209. Shaw, D.V. and R.W. Allard. 1982.

Estimation of outcrossing rates in Douglas-fir using isozyme markers. *Theor. Appl. Genet.* 62: 113-120.

Seeds produced under open-pollination were collected from eight natural stands and a plus-tree seed orchard of Douglas-fir. These seeds were germinated and both diploid embryos and haploid gametophytes were analyzed by starch-gel electrophoresis. Eleven variable loci were resolved for both kinds of tissue and used as genetic markers for estimating outcrossing rates. Estimates made with single-locus and multilocus methods both indicated that the proportion of viable embryos resulting from outcrossing is about 0.90 for the natural stands, and for the seed orchard. Comparison of single-locus and multilocus estimates of outcrossing rates indicated that little or no inbreeding other than selfing occurred. Es-

timated outcrossing rates were higher for seeds from the upper portion of the crown than for seeds from the lower crown. It was also found that some trees selfed at a much higher rate than other trees.

210. Shaw, D.V. and R.W. Allard. 1982.

Isozyme heterozygosity in adult and open-pollinated embryo samples of Douglas-fir. *Silva Fenn.*, 16: 115-121.

Eleven electrophoretically detectable loci were used as markers to examine patterns of heterozygosity in both adults and open-pollinated progenies of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Heterozygotes were less frequent in samples of embryos than expected under panmixia—an observation that is consistent with previously estimated outcrossing rates. Conversely, a slight excess of heterozygotes was found in adults relative to expected panmictic proportions. Most of the differences between the adult and the offspring genotypic distributions probably result from viability selection favoring outcrosses i.e., by selective removal of selfed offspring prior to reproductive maturity. However, viability difference between selfs and outcrosses cannot be responsible for the excesses of heterozygotes observed in the adult samples. Several mechanisms that might account for the excesses are discussed.

211. Shen, H.-H.; D. Rudin and D. Lindgren. 1981.

Study of the pollination pattern in a Scots pine seed orchard by means of isozyme analysis. *Silvae Genet.* 30: 7-15.

A genotype analysis of three loci — LAP-B, GOT-A and B, using some two thousand seeds originating from three ramets of a marker clone and ten grafts from surrounding clones was carried out in a seed orchard close to Umeå. A significant difference with regard to frequency of genotypes from seeds derived from the three ramets was found, and there was variation among sectors at different levels of a crown within a ramet due to differences in the fertilizing

pollen cloud. Pollen dispersal seems to be dependent on the coincidence of the time of flowering of male and female trees, the distance from a pollen tree to a mother tree as well as wind direction during flowering. A neighboring tree with all favorable conditions received 31 percent of the fertilizing pollen from the marker ramet. The average frequency of self-pollinated seeds from the marker clone was roughly estimated at 6 percent with the lowest frequency at the top of the crown.

212. Simonsen, V. and H. Wellendorf. 1975.

Some polymorphic isoenzymes in the seed endosperm of Sitka spruce [*Picea sitchensis* (Bong.) Carr.]. Forest Tree Improvement, 9: 5-20.

Using starch-gel electrophoresis several isoenzymes are found in the endosperms of Sitka spruce seeds. Malate dehydrogenase (MDH) shows seven patterns in the zymogram and only one deviation from expectation is observed when testing one-to-one segregation. Phosphoglucose isomerase (PGI) shows three patterns of the zymogram and no significance when testing one-to-one segregation. Phosphoglucomutase (PGM) has two loci with two and three alleles, respectively. Leucine aminopeptidase (LAP) has two loci with three and four alleles, respectively, one of the alleles of each locus being a silent one. No linkage between the two LAP-loci and the two PGM-loci is found.

213. Smith, D.B. and W.T. Adams. 1983.

Measuring pollen contamination in clonal seed orchards with the aid of genetic markers. In: Proceedings of the 17th Southern Forest Tree Improvement Conference. June 1983.

The availability of electrophoretic techniques has provided a large number of simply inherited genetic markers (allozymes) which can be used to estimate levels of pollen contamination in seed orchards. Using several allozyme loci, the multilocus genotypes of both the male and female gametes contributing to the conifer

embryo can be determined. Because natural stands contain many more adult genotypes than do clonal orchards, they are expected to produce a much greater variety of multilocus pollen genotypes, even when there are no differences in single locus allele frequencies between orchards and background stands. The level of pollen contamination in an orchard can be estimated by analyzing a sample of orchard seed and determining the proportion of pollen gametes having multilocus genotypes which could not be produced in the orchard. This proportion is then divided by the probability that a background pollen grain has a multilocus marker (i.e., a genotype different from those that can be produced by orchard clones) to compute the level of contamination. Estimates of pollen contamination in several blocks of a Douglas-fir seed orchard in western Oregon are used to illustrate this multilocus technique. Preliminary recommendations for its use are also discussed.

214. Snyder, E.B. and J.M. Hamaker. 1978.

Inheritance of peroxidase isozymes in needles of loblolly and longleaf pines. *Silvae Genet.* 27: 125-129.

Progeny from diallel matings were examined by electrophoresis. Eleven different isozymes were observed, nine of which were common to both species. Diallel matings facilitated determining inheritance. The isozyme segregation frequencies approximated Mendelian expectation as did intensity segregants for one loblolly isozyme. Segregation frequencies for three allozymes varied according to the genetic background of certain parents. The segregation of three loblolly and five longleaf isozymes could be attributed to polymorphic loci.

215. Steinhoff, R.J.; D.G. Joyce and L. Fins. 1983.

Isozyme variation in *Pinus monticola*. *Can. J. For. Res.* 13: 1122-1131.

Seeds from 28 stands representing most of the range of *Pinus monticola* Dougl. were analyzed for electrophoretically demonstrable variation in

10 proteins encoded by 12 genetic loci. On the average, 65% of the loci per stand were polymorphic, and expected heterozygosity of offspring was 18%. The populations could be assigned to two geographic groups, a broad northern one and a rather restricted southern one. The southern group consisted of populations from Sierra Nevada and southern Cascade Mountains in northern California and from the Warner Mountains in south-central Oregon. These southern populations were similar to each other but all differed from those of the northern group in allelic frequency patterns for several isozymes. Across the northern part of the species' distribution (British Columbia, Washington, northern Oregon, Idaho, and Montana) differences among stands were minor and essentially random. Collections from stands in the central and southern Oregon Cascades and the Siskiyou Mountains in northwestern California were more nearly like the northern stands but exhibited some characteristics indicative of a transition area between the Sierra and northern types.

216. Sutherland, J.R.; U. Rink; E.E. McMullan and T.A.D. Woods. 1981.

Isozyme characteristics of *Caloscypha fulgens* infested and pathogen-free spruce seed samples and use of alkaline phosphatase activity for qualitative and quantitative disease incidence assays. Can. J. For. Res. 11: 200-205.

Extracts of samples from *Caloscypha fulgens* infested and noninfested Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seed lots and diseased and healthy seeds were separated by electrophoresis on polyacrylamide gels and stained for esterase (EC 3.1.1.1), leucine aminopeptidase (EC 3.4.1.1), acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1), glucose-6-phosphatase (EC 3.1.3.9), malate dehydrogenase (EC 1.1.1.37), isocitrate dehydrogenase (EC 1.1.1.42), 6-phosphogluconate dehydrogenase (EC 1.1.1.43) and alcohol dehydrogenase (EC 1.1.1.1). Several differences were detected between the isozyme patterns of disease-free and infested samples, but the main difference was the latter's high alkaline phosphatase activity. By using gel electrophoresis, (i) a qualitative analysis was developed, based on the presence or absence of alkaline phosphatase, to distinguish in-

festes from disease-free seed lots, and (ii) the high alkaline phosphatase activity of infested samples was determined to be of pathogenic origin. By determining the alkaline phosphatase activity of samples with known numbers of diseased seeds, a prediction equation was derived relating enzyme activity and disease incidence. Correlation analyses showed significant ($P = 0.01$) correlations between disease incidence estimates obtained by this technique and plating of surface-sterilized seeds on water agar.

217. Szmidi, A.E. 1979.

Inheritance of catalase multiple forms in Scots pine (*Pinus sylvestris* L.). Arbor. Kornickie, 24: 105-110. [Genetics Abstracts. G12, 14884].

Starch gel electrophoresis was used to assay for catalase polymorphism in the haploid endosperm of dormant seeds of *Pinus sylvestris*. Seven different catalase variants consisting of 1 to 5 bands were detected. Only 1 or 2 mutually exclusive catalase variants could be found in seeds from each of 155 investigated trees. Statistical analysis of the catalase variant segregation patterns in the seed samples from individual heteromorphic trees revealed no deviations from the expected 1:1 segregation ratio for a 1-locus/2-alleles system. It could be postulated therefore, that catalase electrophoretic variants which occur in Scots pine and endosperm are coded for by 7 alleles at 1 locus.

218. Szmidi, A.E. 1980.

Genetic variation among eleven populations of Scots pine (*Pinus sylvestris* L.) as determined by catalase polymorphism. Arbor. Kornickie, 25: 181-187. [Genetics Abstracts G13, 9179].

The catalase polymorphism was studied in 11 populations of *P. sylvestris*. 3-6 different catalase variants occurred in each of the investigated populations. The observed heterozygosity ranged between 0.350 and 0.600. Highest heterozygosity was observed in Strzawo, Milomlyn, Bolewice and Babki populations, the most valu-

able pine stands in Poland. On the other hand, southern populations considered as poorly growing were less heterozygous.

219. Szmidt, A.E. 1982.

Genetic variation in isolated populations of stone pine (*Pinus cembra*). *Silva Fenn.*, 16: 196-200.

Allozyme variation at 8 enzyme loci (Lap-1, Lap-2, Got-1, Got-2, APh-1, ADh-1, Cat-1 and Est-2) was investigated by means of starch gel electrophoresis and gel isoelectric focusing in 11 populations of stone pine. Average values of expected panmictic heterozygosity (H) were relatively lower than those recorded in populations of other conifers and ranged between 0.165 and 0.397. Considerable differences were found between investigated populations with regard to the frequency of occurrence of analyzed allozymes. Values of Nei's genetic distance (D) were very high and ranged between 0.018 and 0.650. Highest values of D were found between Asiatic population Czita in comparison with the remaining populations. According to a dendrogram based on the calculated D values the investigated populations could be divided into several groups corresponding to their geographic origin.

220. Tajima, M.; H. Miyajima and Y. Miyazaki. 1977.

Genetic analysis for peroxidase isozymes in *Chamaecyparis obtusa* Endl. *Journal of the Japanese Forestry Society*, 59: 173-177. [Forestry Abstracts 40, 538].

Peroxidase variation was studied in 13 Japanese clones. The inheritance of the isozyme bands was examined in a 4 X 4 complete diallel cross.

221. Thiebaut, B.; Lumaret, R. and Vernet, P. 1982.

The bud enzymes of beech (*Fagus sylvatica* L.) genetic distinction and analysis of polymorphism in several French populations. *Silvae Genet.* 31: 51-60.

This paper describes the methods used to study enzymatic genetic markers in Beech (*Fagus sylvatica* L.), and indicates the reasons which led us to work particularly on buds.

Starch gel electrophoresis was used to study the genetics of 3 loci, two coding for peroxidases (Px1 and Px2) and one coding for a glutamate-oxaloacetate transaminase (GOT₁), in Beech buds. The peroxidases are monomeric enzymes in both cases, the first locus is composed of 2 alleles Px₁^{1.00} and Px₂^{1.05} (with existence of silent alleles in certain cases) and the second of 3 alleles Px₂^{1.13}, Px₂^{0.26}, Px₂^{0.40}. The glutamate-oxaloacetate transaminases are dimeric enzymes specified by one locus composed of two alleles: GOT₁^{1.00} and GOT₁^{1.05}.

Using 11 Southern and 2 Northern populations, polymorphism is examined simply for the peroxidases (2 loci). The variation of allelic frequencies show clearly the influence of climate; in the first locus the moisture regime seems to be involved, and in the second the temperature regime.

222. Tigerstedt, P.M.A. 1973.

Studies on isozyme variation in marginal and central populations of *Picea abies*. *Hereditas*, 75: 47-60.

Two delimited populations of *Picea abies*, each comprising about 50 individuals have been analyzed with regard to three different enzyme systems, esterase (EST), leucine-amino-peptidase (LAP) and acid phosphatase (PHOS).

Female gametophytic endosperm tissue dissected from mature seed has been used for all three isozyme analyses. As this tissue is haploid, a novel method for distinguishing heterozygotes and homozygotes has been used.

The spatial mapping of genotypes in their habitat indicates complete randomness of gene distribution within stands.

In two cases (LAP and PHOS), enzyme analyses also indicate strict conformity of allozymes to expected Hardy-Weinberg frequencies. Allele frequencies in the range of 0.3-0.7 for two or more allozymes suggest heavy gene flow, frequency-

dependent selection or, possibly, founder effects. In the third enzyme system analysed (EST), there is indication of balanced heterozygosity.

There appears to be plenty of genetic variability of allozymes even at the species margin. The adaptive significance of different enzyme systems is briefly discussed.

223. Tigerstedt, P.M.A. 1974.

Genetic structure of *Picea abies* populations as determined by the isozyme approach. In: Proceedings, Joint IUFRO Meeting, S.02.04.1-3, Stockholm, 1974, Session V. pp. 283-291.

Ten populations of *Picea abies* have been analysed for genetic structure using the isozyme separation technique. Leucine-amino-peptidases, esterases, acid phosphatases and alcohol dehydrogenases were electrophoretically analysed.

Allozyme systems conformed astonishingly well with the Hardy-Weinberg equilibrium.

Gene frequencies along the latitudinal gradient (60 — 69°N) suggested robust or flexible polymorphisms or gene neutrality in different allozyme systems.

Overall genetic heterozygosity was astonishingly high even in marginal populations. Enzyme systems differed in the degree of heterozygosity correlated with the latitudinal gradient.

It is concluded that different enzyme systems react differently under the pressure of natural selection.

Picea abies seems to have a lot of genetic flexibility in its populations. This flexibility is maintained through a unique species strategy and genetic system; high crossing over frequency, little supergene formation, possibly gene duplication, heavy gene flow and hence an array of different genotypes offered to natural selection in each generation.

224. Tigerstedt, P.M.A.; D. Rudin; T. Niemela, and J. Tammissola. 1982.

Competition and neighboring effect in a naturally regenerating population of Scots pine. *Silva Fenn.*, 16: 122-129.

A natural pine stand, 300-400 years old, and a progeny generation underneath, about 100 years old, were analysed for population structure by means of isozyme analysis. The genetic structure of old growth conformed to Hardy-Weinberg equilibrium and neighboring trees appeared on the average to be slightly related. The genetic structure of regeneration departed significantly from Hardy-Weinberg equilibrium and appeared more inbred with high proportions of homozygotes. Regeneration groups around old growth trees did not appear to be particularly related to the tree above. It is suggested that seeds in natural stands move in cohorts and land in cohorts thus mixing up the population structure. It is also suggested, that selection against high proportions of inbreds in seedling populations is important to avoid inbreeding depression in natural populations.

225. Tobolski, J.J. 1978.

Isozyme variation in several species of oaks. In: Proceedings of the Second Meeting of the Central Hardwood Forest Conference, Purdue University, pp. 456-468.

This investigation analyzed the isozyme patterns from bud and acorn cotyledon tissues of one or more seed sources of northern red oak, black oak, white oak, swamp white oak, bur oak and chinkapin oak. Isozyme patterns were determined for leucine aminopeptidase, alcohol dehydrogenase, acid phosphatase and glutamic-oxaloacetic transaminase.

Most isozyme bands were found to be common to two or more of the species investigated. This isozyme similarity appears to reflect their close phylogenetic relationship. However, the individuals of some species exhibited distinctive phenotypes and they could be readily distinguished from other species.

226. Tobolski, J.J. 1979.

Inheritance of polymorphic isozymes in jack pine (*Pinus banksiana* Lamb.). In: Proceedings of the First North central Tree Improvement Conference, Madison, Wisconsin, August 21-23, 1979. pp. 147-154.

The inheritance of 7 polymorphic enzymes was determined by starch gel electrophoresis of megagametophyte tissue from 33 seed sources of jack pine. Twenty-nine isozyme variants encoded by 10 loci were observed. The enzymes glutamic-oxaloacetic transaminase and malate dehydrogenase displayed a fast anode-migrating zone of activity with two segregating variants and a slower zone with 3 segregating variants. For alcohol dehydrogenase and glutamate dehydrogenase three segregating variants were recovered. Four segregating variants were recovered for 6-phosphogluconate dehydrogenase, acid phosphatase and leucine aminopeptidase. Also a segregating null variant was observed in a faster zone of leucine aminopeptidase. Segregation patterns suggest that each of these variants is under the control of single locus.

227. Tobolski, J.J. and M.T. Conkle. 1977.

Enzyme identification of Austrian $\times$ Japanese red pine hybrids in seed from mixed pollen controlled crosses. In: Proceedings of the 10th Central States Forest Tree Improvement Conference, Sept. 22-23, 1976. Purdue University, West Lafayette, Indiana. pp. 35-41.

Mixed pollen samples with 10:90, 50:50, and 90:10 percent of *Pinus densiflora* and *P. nigra* pollen respectively were used to control pollinate *P. nigra* seed trees. The test was undertaken to determine if hybrids were produced in proportion to *P. densiflora* pollens in the mixes. Offspring embryos were electrophoresed and hybrid offspring were identified using alcohol dehydrogenase alleles that differed between the two species. In all three pollen-mix treatments, hybrid production was significantly reduced over that expected from the ratio of *P. densiflora* species pollen in the mix. With low and moderate concentrations of *P. densiflora* low frequencies of hybrids are produced. The pollen mix had to con-

tain high concentrations of *P. densiflora* pollen before significantly large proportions of hybrids were produced. The data supports the hypothesis that native embryos are highly favored over hybrid embryos as judged by the proportion of hybrids in the resulting seed. The results are discussed briefly with reference to the suggestion that hybrids can be mass produced by natural fertilization of mixed plantings of *P. nigra* and *P. densiflora* trees.

228. Tsay, R.C. and I.E.P. Taylor. 1978.

Isoenzyme complexes as indicators of genetic diversity in white spruce, *Picea glauca*, in southern Ontario and the Yukon Territory. Formic, glutamic, and lactic dehydrogenases and cationic peroxidases. Can. J. Bot. 56: 80-90.

A center of genetic diversity for white spruce, *Picea glauca* (Moench) Voss, is suspected in and around a refugial region in the Yukon Territory. Seed from the refugium (30 samples), a neighboring region (42 samples) and from southern Ontario (50 samples) was examined for isoenzymes of formic (FDH), glutamic (GDH), and lactic (LDH) dehydrogenases and cationic peroxidases (CP). FDH patterns were uniform. There were three GDH bands which appeared in five patterns. Eleven isoenzymes of LDH were detected in 21 patterns. There were 25 CP bands and the pattern from each collection (containing 7-13 bands) was unique.

The results from GDH, LDH, and CP analyses revealed differences between seed from the refugium and the adjoining regions. The patterns for LDH supported the hypothesis that the refugium was a center of diversity but the GDH and CP patterns were more diverse outside the refugium. The LDH and CP analyses showed that the genetic resources of the two regions in the Yukon were more diverse than those in southern Ontario. Analysis of divergence showed that these differences were significant at the 1% level of probability.

229. Vidgren, J. and M. Hagman. 1982.

Variation in the activity of the catalase enzyme in provenances of Scots pine. *Silva Fenn.*, 16: 189-195.

The variation in the activity of the catalase enzyme was studied in young seedlings of Scots pine (*Pinus sylvestris* L.) grown from 41 different samples from the USSR and from 10 samples of Finnish origin. The activity of the enzyme expressed per milligram fresh weight of seedlings seems in general to decrease with decreasing latitude of origin between latitude 67°N. and 54°N. Below the latitude 54°N. this trend is not so clear. In all samples studied there is a considerable variation between the individual seedlings. The possibilities of using the activity of the catalase enzyme in distinguishing between provenances of Scots pine and in the selection of northern and southern types within a provenance are discussed.

230. Wang, T.T.; C. Kao; J.C. Yang and J.G. Shyu. 1976.

Isoenzyme variation of peroxidase in *Taiwania cryptomerioides* Hay. Bulletin, Experimental Forest, National Taiwan University. 118: 1-15. [Forestry Abstracts 39, 1195].

Isoenzyme variation was studied in leaf samples from various parts of Taiwan by electrophoresis, using similar techniques to those previously reported. There were no significant differences between samples from different areas, or from different parts of the tree. The patterns varied with age and from year to year; activity was lowest in years of greatest biological production.

231. Weber, J.C. and F.R. Stettler. 1981.

Isoenzyme variation among ten populations of *Populus trichocarpa* Torr. et Gray in the Pacific Northwest. *Silvae Genet.* 30: 82-87.

Isoenzyme variation was analyzed among ten riparian populations of black cottonwood in the Pacific Northwest. Isoenzymes from 12 enzyme systems were extracted from root tips of rooted

cuttings and separated by horizontal starch-gel electrophoresis.

Although the populations were geographically distinct, overall genetic similarity was very high and average within-population heterozygosity (H_S) was much greater than between-population heterozygosity (D_{ST}). Neighboring populations clustered together genetically, but no statistically significant relationship existed between geographic and genetic distance. Gene diversity, measured by both average heterozygosity per locus and the proportion of polymorphic loci, was generally greater in populations representing larger river drainages, although the differences were very slight. The frequencies of four alleles varied with latitude or longitude, but other allele frequencies appeared unrelated to those coarse gradients. Nearly 13% of the loci sampled did not fit Hardy-Weinberg proportions; most deviations were due to a deficiency of heterozygotes.

Results are discussed in terms of potential factors promoting genetic similarity and differentiation among natural populations of black cottonwood.

232. Wellendorf, H. and V. Simonsen. 1979.

A chemotaxonomic study in *Picea* with isoenzymes in the seed endosperm. In: Proceedings of the Conference on Biochemical Genetics of Forest Trees, Umea, Sweden 1978. pp. 182-193.

The aim of our chemotaxonomic work in *Picea* is to obtain an impression of the true relations of the score of species in this genus. Such information is of interest in species crossing projects, but might as well give valuable spin-off information of evolutionary interest.

In the seed endosperm 7 isoenzyme systems are analyzed using starch-gel electrophoresis. Each system is determined typically by 2-4 loci, each with 3-6 alleles — that means the comparison of species is based on 71 alleles. In each species 50 seeds are analyzed in each of typically 2-3 well separated geographic samples areas (provenances). From the 21 analyzed species a binary data set is built up. Each observation is one species (pooled samples), and the 71 alleles are scored as either present or absent (1 or 0). On the basis of

this similarity matrices are computed and cluster analyses are attempted.

233. Wendel, J.F. and C.R. Parks. 1982.

Genetic control of isozyme variation in *Camellia japonica* L. *J. Hered.* 73: 197-204.

Procedures are described for the extraction and electrophoretic separation of enzymes from seed and leaves of the tannin-rich evergreen *Camellia japonica* L. Seventeen enzyme systems have been resolved, and of these all but three were polymorphic. Crosses were performed between parents bearing dissimilar allelomorphs in order to discern the genetic control of the resolved enzymes. Based on the segregation ratios observed, 12 of the zones of staining for eight enzyme systems were postulated to be codominantly inherited single-gene traits. Tests of joint segregation for 58 out of 66 possible pairwise combinations for the 12 postulated loci suggested that two pairs of genes are linked: AAT-1 with PGM-3 ($r = 0.29 \pm 0.03$), and 6-PGD-2 with PGM-2 ($r = 0.17 \pm 0.02$).

234. Wheeler, N.C. and R.P. Guries. 1982.

Biogeography of lodgepole pine. *Can. J. Bot.* 60: 1805-1814.

Lodgepole pine has been a component of the western North America flora throughout recent geological history although its present widespread distribution may be a relatively recent phenomenon (late Pleistocene). The differentiation of populations within and between subspecies, and the distribution of rare alleles, both suggest that at the time of maximal Wisconsin glacial advance lodgepole pine was restricted to a number of refugia in the western United States, the islands along the north Pacific coast, and in an unglaciated region of the west central Yukon. Subsequent to glacial retreat, lodgepole pine migrated to its present range with northern and southern interior populations meeting in northern British Columbia, possibly within the last 4 000 years.

235. Wheeler, N.C. and R.P. Guries. 1982.

Population structure, genic diversity, and morphological variation in *Pinus contorta* Dougl. *Can. J. For. Res.* 12: 595-606.

Lodgepole pine (*Pinus contorta* Dougl.) occupies a large and ecologically diverse range in western North America. The species is generally recognized as consisting of four subspecies, three of which are widespread and the fourth endemic to the north coast of California. Geographic isolation of subspecies is nearly complete although gene flow may occur in certain instances. Moderate heterozygosity estimates (0.10-0.14) and low standard errors of the estimate (0.02-0.04) were obtained for all populations of lodgepole pine and are comparable with other recent studies of conifers that based estimates on 20 or more loci. The distribution of allozyme variation indicates that very little population differentiation has occurred. Approximately 91% of the allozyme variability resides within populations, with 3 and 6% distributed among subspecies and among populations, respectively. The extent of population differentiation is far less than that observed for many herbaceous plants but similar to reports for other conifers. In contrast, a large proportion of morphometric variation in lodgepole pine cone and seed traits is distributed among subspecies (38%) and among populations within subspecies (19%). This may be attributed to the effects of strong selective pressures acting on important reproductive structures and to the conventional methodology for analyzing morphometric variation. The present study confirms, with minor exceptions, an earlier taxonomic treatment of lodgepole pine. Notably, the assignment of lodgepole pine in the Cascade Range of southern Oregon to ssp. *murrayana* is probably inappropriate. While morphological data suggest a close affinity to ssp. *murrayana*, allozyme data suggest that populations in this area should be assigned to ssp. *latifolia*.

236. Wheeler, N.C. and R.P. Guries. 1983.

Hybridization and introgression between lodgepole pine and jack pine: detection and measurement. *Evolution*. 37: in press.

A method of data analysis commonly used by human geneticists has been used for the study of introgression in long-lived forest trees. Gene exchange between taxa is estimated by m , the proportion of genes in a population attributable to an introgressant population. Analyses of gene frequency data for 42 loci in 38 populations of jack and lodgepole pine and their putative hybrids suggests that reciprocal gene exchange may be common following hybridization events but may be of a limited or transient nature. More intensive sampling is necessary to determine the actual limits of localized gene exchange. The widespread or dispersed introgression apparent in populations in the Yukon and western U.S. seems to result not from long-distance pollen or seed dispersal, but rather from ancient contact between jack and lodgepole pines. Historical factors, especially glaciation, migration, and the creation of disturbed habitats following glacial recession, probably account for the present day occurrence of jack pine influence far from the present-day range of jack pine. Anderson's (1949) classic approach to the study of introgression using hybrid indices appears to provide a relatively accurate accounting of gene exchange between taxa. Numerical estimates of introgression based on genetic and morphological data bases were significantly correlated ($r = .98$, $p < .01$).

237. Wheeler, N.C.; R.P. Guries and D.M. O'Malley. 1983.

Biosystematics of the genus *Pinus*, subsection *Contortae*. *Biochem. Syst. Ecol.*, 11: 333-340.

Gene frequency data from 42 isoenzyme loci were used to assess the phylogeny of taxa in the genus *Pinus*, subsection *Contortae*. The classification inferred from a phenogram based on Nei's genetic distance measure (D_N) was generally in good agreement with the current taxonomic treatment of the subsection. *Pinus contorta* appears to most closely resemble the ancestral taxon from which all other taxa in the subsection are derived. An alternative classification based upon Mahalanobis squared distances (D_R) obtained from seed and cone measurements differed from the isoenzyme treatment by placing *P. contorta* subsp. *murrayana* in close association with *P. virginiana*.

238. Witter, M.S. and P.P. Feret. 1978.

Inheritance of glutamate oxalo-acetate transaminase isozymes in Virginia pine megagametophytes. *Silvae Genet.* 27: 129-134.

Polyacrylamide gel electrophoresis was used to examine inheritance of GOT isozymes in megagametophytes of Virginia pine. Two unlinked loci were found to code for GOT: Locus A with 5 alleles, and Locus B with 3 alleles. Irregular segregation patterns were found among megagametophyte populations of some trees. These results suggest the possibility of non-random degeneration of 3 of 4 megaspores produced in meiosis.

Four populations of Virginia pine were compared on the basis of allelic frequency and occurrence. Little or no significant differences were found.

239. Witter, M.S. and P.P. Feret. 1979.

Inheritance of esterase and acid phosphatase isozymes in Virginia pine and the application of the isozyme technique to a seed orchard population. *Silvae Genet.* 28: 213-220.

The inheritance of esterase and acid phosphatase isozymes in Virginia pine (*Pinus virginiana* Mill.) was determined by an analysis of the segregation patterns among the megagametophytes of seed collected from individual trees. Both esterase and acid phosphatase phenotypes consisted of multiple isozymes which segregated in a 1:1 ratio, consistent with the behavior of allelic variants at a single gene locus. The esterase phenotypes were unusual. The variants were usually not characterized by differences in isozyme mobility, but instead, segregated for differences in staining intensity. Acid phosphatase segregation patterns indicated control by a minimum of two independent loci. Although genetic control of the two enzyme systems was demonstrated, allelic designations were not assigned to each phenotype. The problems associated with an allelic interpretation when phenotypes consist of multiple isozymes are discussed.

A seed orchard population and a natural population of Virginia pine were analyzed in terms of the three enzyme systems (EST, APH, GOT) described for Virginia pine. The measurement of

the effects of artificial selection on the genetic structure of forest tree populations using the isozyme technique is discussed.

240. Woods, J.H.; G.M. Blake and F.W. Allendorf. 1983.

Amount and distribution of isozyme variation in ponderosa pine from eastern Montana. *Silvae Genet.* 32: 151-157.

Pinus ponderosa seeds from 50 trees in each of six small isolated stands located within a nine kilometer radius of Colstrip, Montana, were examined at 23 isozyme loci. Megagametophyte and embryo tissue from each seed were screened separately. Measures of genetic diversity showed no significant differences between male and female components within or between stands. The average proportion of heterozygous loci per embryo (H_e) was not significantly different between stands. For all stands combined, H_e was 0.012. Genotype proportions did not deviate from expected Hardy-Weinberg proportions.

Nearly 99% of the genetic diversity resided within individual stands, with a significant 1.5% due to differences between stands. Genetic distance between stands is not correlated with geographic distance. Considering the natural fire history of the stands, it is suggested that there is a great deal of gene flow into a stand during its early stage of development.

241. Yang, J.C. 1976.

Isoenzyme variation of coastal Douglas-fir. [*Pseudotsuga menziesii* (Mirb.) Franco. var. *menziesii*]. II. Genotype — environment relationship in nine provenances. *Quarterly Journal of Chinese Forestry*, 9: 77-89. [Forestry Abstracts 38,4670].

Young germinants of 9 provenances were sampled from seedlots in Vancouver Is., Washington, Oregon and California. Climatic data, and allele frequency data from 4 polymorphic loci of 3 enzyme systems, were analyzed. Large differences in allele frequencies were observed between the 9 provenances. With one exception, at

least one component of each locus was significantly correlated with one or more environmental factors, the most important being latitude and altitude. These results are consistent with the theory of natural selection for the maintenance of isoenzyme polymorphisms in Douglas-fir, rather than with functional neutrality.

242. Yang, J.C.; T.M. Ching and K.K. Ching. 1977.

Isoenzyme variation of coastal Douglas-fir. I. A study of geographic variation in three enzyme systems. *Silvae Genet.* 26: 10-18.

Genetic variability in natural populations of Douglas-fir was studied at the enzymatic level. By the techniques of disc gel electrophoresis and biochemical staining, the isoenzyme patterns of leucine aminopeptidase (LAP), esterase (EST), and glutamate oxaloacetate transaminase (GOT) were characterized in young seedlings at the stage when cotyledons were completely elongated. Samples of 107 seedlots were collected from nine provenances located at Vancouver Island, Washington, Oregon, and California.

Allelic frequency differences are statistically significant among the provenances examined, but appear to be rather complex. By contrast, variation in genic heterozygosity exhibited some geographic uniformity of clinal changes. The combined effect of gene flow and some form of balancing or directional selection was considered as the probable mechanism that maintains uniformity or clinal variation of allelic heterozygosity.

The decreased proportions of heterozygotes at EST-B and GOT-B loci were considered to be associated with negative effects of some selective pressures imposed by edaphic or climatic factors, such as soil type and moisture stress in the summer.

Genetic differentiation in terms of genetic identity and genetic distance between provenances of Douglas-fir is more or less parallel to the geographic distance.

243. Yazdani, R. and D. Rudin. 1982.

Inheritance of fluorescence esterase and β -galactosidase, in haploid and diploid tissues of *Pinus sylvestris* L. *Hereditas*, 96: 191-194.

Starch gel electrophoresis was used to study the pattern of inheritance at both the fluorescence esterase (F-EST) and the β -galactosidase (β -GAL) loci in the macrogametophyte, bud and needle tissues of *Pinus sylvestris*. The segregation patterns of these two enzymes were tested in the progeny resulting from 10 different controlled crosses. The result of this study is compatible with the distribution pattern expected for simple single-locus inheritance. Four alleles were identified at the F-EST locus, and two alleles at the β -GAL locus. Both F-EST and β -GAL appear to be dimeric enzymes.

244. Yeh, F.C. 1979.

Analyses of gene diversity in some species of conifers. In: Proceedings of Symposium on Isozymes of North American Forest Trees and Insects, July 27, 1979, Berkeley, Calif. Gen. Tech. Rep. PSW-48. Pacific Southwest Forest and Range Exp. Sta., Forest Serv., U.S. Dep. Agric., Berkeley, Calif., pp. 48-52.

Genetic variation at 21 to 25 loci in extracts of individual megagametophytes was surveyed in Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), Sitka spruce (*Picea sitchensis* [Bong.] Carr.) and lodgepole pine (*Pinus contorta* spp. *latifolia* [Engelm.] Critchfield). The overall mean proportion of polymorphic loci was 61.19 percent, and the overall mean heterozygosity per individual was 15.81 percent. Sitka spruce was on the low side and interior Douglas-fir on the high side of overall mean genetic variation. Distribution of loci relative to frequency of heterozygotes was rather even for heterozygosities between 0.05 and 0.60; however, between 38 to 56 percent of the loci had heterozygosities lower than 0.05. More than 90 percent of the total gene diversity resided within populations. Although subpopulations were differentiated by only 2.6 to 7.9 percent, level of population subdivision was considered significant for the species tested. The overall pattern of genetic differentiation agreed

with the expected on the basis of the neutral-mutation theory. Some loci, however, demonstrated conspicuous clinal variation patterns that are not readily compatible with this stochastic model.

245. Yeh, F.C. 1979.

The role of isozyme research in tree improvement. In: Proceedings 17th Meeting of the Canadian Tree Improvement Association. Symposium: Tree Improvement in the Boreal Forest: Today and Tomorrow. pp. 101-108.

Gel electrophoresis is an efficient tool for large-scale genetics investigations. It is particularly valuable to tree breeders and forest geneticists since its unique features of simple inheritance and small environmental effects offer excellent opportunities to increase our knowledge of the genetic relationships between and within species of coniferous trees and to solve problems encountered in tree breeding and genetics research. These include identifying subpopulations, relatives and seed origin, determining the effectiveness of mass pollination techniques and the degree of selfing and wild pollen contamination in seed orchards, and aiding breeding programs through indirect selection.

246. Yeh, F.C.; A. Brune; W.M. Cheliak and D.C. Chipman. 1983.

Mating system of *Eucalyptus citriodora* in a seed-production area. *Can. J. For. Res.* 13: 1051-1055.

Arrays of open-pollinated seeds were assayed for allozyme polymorphisms at three loci (Adh, Gdh, and Mdh-2) to obtain a quantitative estimate of outcrossing rate in a seed-production area of *Eucalyptus citriodora* (Hook). Rate of outcrossing varied among loci, but suggested overall that up to 14.7% of the seed might be derived from self-fertilization at the viable embryo stage. Such a level of partial self-fertilization, together with local variation in the mating system, yielded an observed average inbreeding coefficient of 0.205 for these loci in the progeny arrays. By contrast, the mature trees exhibited substantial

excesses of heterozygotes ($F = -0.300$), concordant with but not necessarily supportive of earlier hypotheses that selection against homozygotes through the life cycle occurred in eucalypts.

247. Yeh, F.C. and Y.A. EL-Kassaby. 1980.

Enzyme variation in natural populations of Sitka spruce (*Picea sitchensis*). I. Genetic variation patterns among trees from 10 IUFRO provenances. Can. J. For. Res. 10: 415-422.

Genetic variation at 24 enzyme loci was studied for 10 IUFRO populations of Sitka spruce (*Picea sitchensis* (Bong.) Carr. The average proportion of polymorphic loci per population was 0.51 ± 0.02 and the average proportion of heterozygous loci per individual was 0.15 ± 0.03 . The following generalizations were advanced; (1) the amount of genetic polymorphism varied considerably from locus to locus; (2) many populations were similar in the amount and pattern of genetic variation for most loci; (3) but some loci had large differences among populations.

Eight percent of the gene diversity was attributed to interpopulation differentiation; 92% of the total gene diversity resided within populations. Pairwise comparisons of genetic distances averaged 0.014, a value which reflected very little differentiation between geographic sources.

248. Yeh, F.C. and C. Layton. 1979.

The organization of genetic variability in central and marginal populations of lodgepole pine *Pinus contorta* spp. *latifolia*. Can. J. Genet. Cytol. 24: 487-503.

A survey was conducted of genetic variation at 25 loci in extracts of individual megagametophytes of lodgepole pine. Collections were made in nine widely separated localities representing four marginal, two intermediate and three central populations. Single populations of lodgepole pine were, on the average, polymorphic at 58.67% of their loci, and had 1.90 alleles per locus. Both expected and observed heterozygosity averaged 0.16. There was a definite trend towards decreased genetic variability at the

margins. The measures of gene diversity for the 25 loci showed a 4% but significant effect of interpopulation differentiation; 96% of the total gene diversity resided within populations. Estimated outcrossing rates ($\bar{i}$) for the nine populations ranged between 0.93-1.29. Comparisons among populations with different levels of outcrossing revealed no clear relationships between ($\bar{i}$) and amount of genetic variability. The overall pattern of genetic differentiation agrees with expectations based on the neutral mutation theory. However, two loci demonstrated conspicuous clinal variation patterns which may be incompatible with this stochastic model.

249. Yeh, F.C. and D.M. O'Malley. 1980.

Enzyme variations in natural populations of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, from British Columbia. I. Genetic variation patterns in coastal populations. Silvae Genet. 29: 83-92.

We have studied genic variation patterns at 21 loci coding for 15 enzymes in eleven natural populations of Coastal Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, from British Columbia. The average proportion of polymorphic loci per population was $68.83 \pm 1.86\%$ and $51.51 \pm 1.78\%$ at the 99% and 95% criterion for polymorphism, respectively. The average proportion of heterozygous loci per individual was $15.46 \pm 3.70\%$. A high degree of inter-locus variation in heterozygosity within populations was revealed. Spatial pattern of allelic frequencies and heterozygosities departed from random at eight loci. The observed patterns were clinal with respect to latitude, longitude and elevation.

Our measurement of gene diversity for the 21 loci showed a 3% significant effect of interpopulation differentiation. Ninety-seven percent of the total gene diversity resided within populations.

The mean genetic distance over all pairs of Coastal Douglas-fir was 0.0037. A product-moment correlation between the genetic distance of populations and the geographic distance between them yielded a significant value, $r = 0.3116$ ($P \leq 0.05$).

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<i>Pinus nigra</i> (Austrian pine)	21, 36, 95-96, 156, 169-171, 227
<i>Pinus oocarpa</i> (ocote pine)	133
<i>Pinus palustris</i> (longleaf pine)	34, 81, 96, 214
<i>Pinus ponderosa</i> Laws. (ponderosa pine)	8, 31, 48, 96, 104, 118-122, 146-150, 175, 240
<i>Pinus pungens</i> (Table - mountain pine)	58
<i>Pinus radiata</i> (Monterey pine)	95-96, 112, 132, 153-154
<i>Pinus resinosa</i> (red pine)	8, 68, 156
<i>Pinus rigida</i> (pitch pine)	4, 48, 74-78, 93, 116, 172, 177
<i>Pinus serotina</i> (pond pine)	34
<i>Pinus strobus</i> (eastern white pine)	46-47, 95
<i>Pinus sylvestris</i> (scots pine)	11, 26, 43-45, 48, 73, 94-95, 107-110, 131, 138, 141, 161-165, 167, 173, 182-185, 187-188, 190-198, 211, 217-218, 224, 299, 243
<i>Pinus taeda</i> (loblolly pine)	4-7, 32-34, 48, 66-67, 69, 81, 83, 93, 96, 177, 186, 214
<i>Pinus thunbergii</i> (japanese black pine)	178, 201-202
<i>Pinus torreyana</i> (Torrey pine)	115

<i>Pinus virginiana</i> (Virginia pine)	34, 64, 237-239
<i>Populus alba</i> (white poplar)	99-100
<i>Populus albuglandulosa</i> (white × glandulosa Hybrid)	100
<i>Populus deltoides</i> var. <i>occidentalis</i> (plains cottonwood)	79, 99
<i>Populus euramericana</i> (European × American Hybrid)	99
<i>Populus glandulosa</i>	99-100
<i>Populus nigra</i> (black poplar)	99
<i>Populus tremula</i> (European aspen)	80
<i>Populus tremuloides</i> (quaking aspen)	24, 145-146
<i>Populus trichocarpa</i> (black cottonwood)	231
<i>Prunus serotina</i> (black cherry)	117
<i>Pseudotsuga menziesii</i> (Douglas-fir)	17, 31-33, 37-39, 48-54, 96, 118, 120, 130, 137, 141, 158, 208-210, 213, 241-242, 244, 249
<i>Pseudotsuga taxifolia</i> (syn. <i>P. menziesii</i>)	96
<i>Quercus alba</i> (white oak)	135-136, 225
<i>Quercus bicolor</i> (swamp white oak)	225
<i>Quercus macrocarpa</i> (bur oak)	225
<i>Quercus muehlenbergii</i> (Chinkapin oak)	225
<i>Quercus petraea</i> (durmast oak)	174
<i>Quercus robur</i> (English oak)	174
<i>Quercus rubra</i> (northern red oak)	90, 225
<i>Quercus velutina</i> (black oak)	225
<i>Robinia pseudoacacia</i> (black locust)	101
<i>Sequoia gigantea</i> (giant sequoia)	65
<i>Shorea leprosula</i> (mahogany)	70
<i>Spirodela polyrrhiza</i> (common ducks meat)	178
<i>Taiwania cryptomerioides</i> (Taiwania)	230
<i>Thuja plicata</i> (western red cedar)	40, 48
<i>Thujopsis dolabrata</i> (Hiba arbor-vitae)	199, 203-205
<i>Ulmus japonica</i> (japanese elm)	57
<i>Ulmus pumila</i> (Siberian elm)	57, 62-63
<i>Ulmus rubra</i> (gray elm)	57
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